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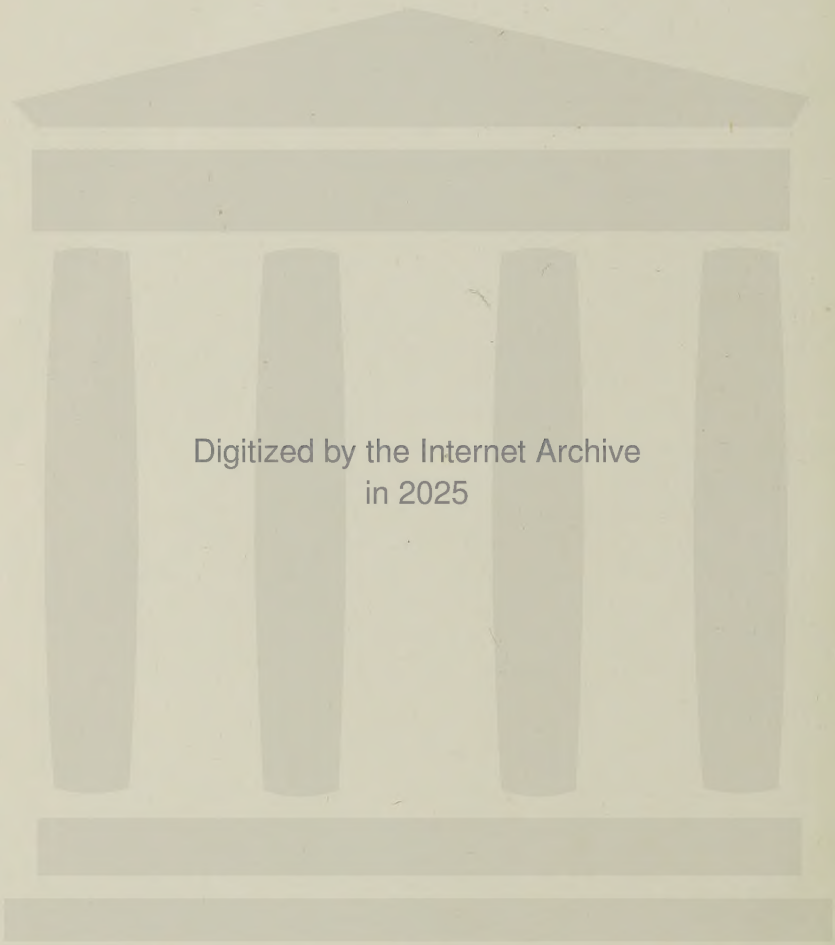
PROFESSOR W. B. BRIERLEY, D.Sc.

For 25 years, Professor Brierley's name has appeared in the list of officers of the Association as Hon. General and Botanical Editor of *The Annals of Applied Biology*. Now that he has felt obliged to relinquish this heavy task, it is fitting that acknowledgement should be made of his great and long-continued services to the Association.

In 1921, when Professor Brierley took over the editorship, the affairs of the Association were somewhat under a cloud and the possibility of continued publication of the *Annals* was in doubt. Since then, the *Annals* has gone from strength to strength and now has a high reputation, both at home and abroad, as a leading biological journal. It has appeared with the utmost regularity, and even during the difficult war years, though delays were inevitable, there was no gap in the series of volumes. The editor has been generous of his time and energy, always with the aim of ensuring a high standard, both from the scientific and literary points of view, for the papers published; no trouble was too great for him to take and no detail too small for careful consideration.

Together with the relief from much hard work, Professor Brierley must feel a sense of loss in no longer piloting the successive numbers of the journal through the press, but he may rest assured that no effort will be spared to maintain and enhance its value to biologists. The science of applied biology and the Association of Applied Biologists in particular owe much to his devoted work and members may best show their gratitude by giving every support to his successor in the editorship of the *Annals*.

C.T.G.



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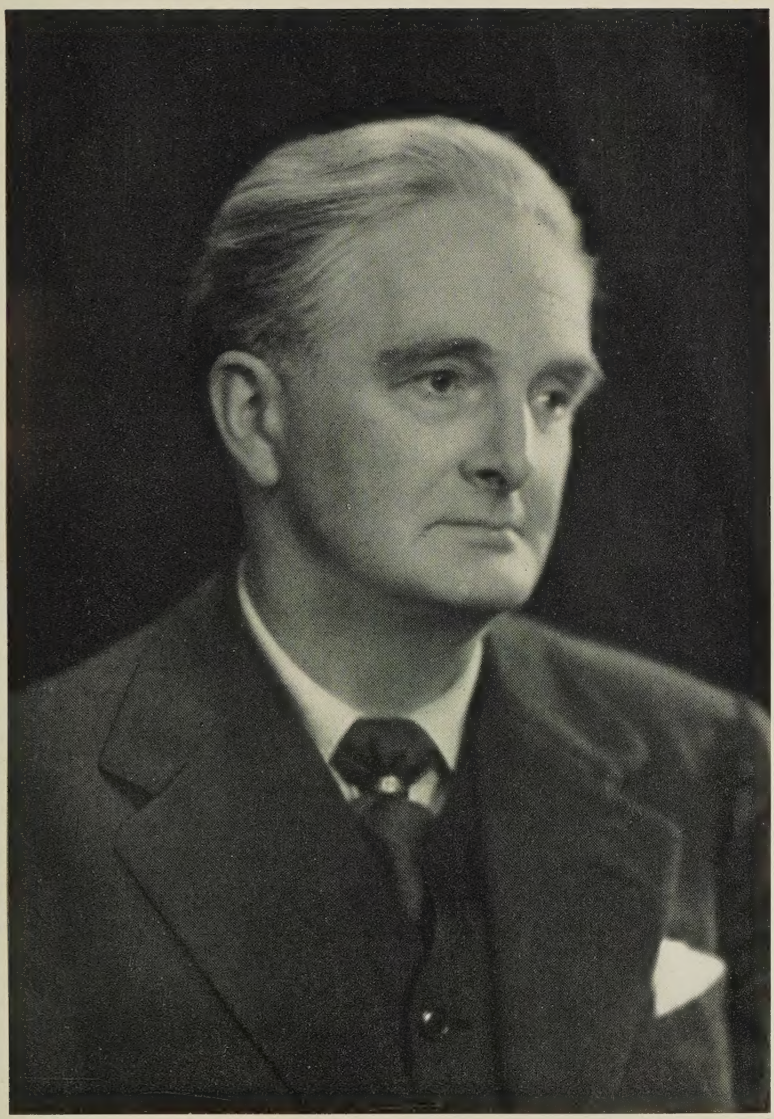


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PROFESSOR W. B. BRIERLEY, D.SC.

Molybdenum as a factor in the nutrition of lettuce

BY KATHERINE WARINGTON, *Botanical Department, Rothamsted Experimental Station*

(With Plate 10 and 2 Text-figures)

Lettuce grown in nutrient solution sometimes showed slight benefit on a dry-weight basis from the addition of 0·1 p.p.m. Cr, Sr, Ti or V, while Zn was usually harmful. Addition of Mo at a similar concentration, however, exerted such a marked beneficial effect on both yield and appearance as to suggest that this element was essential for healthy growth. Confirmation of the response was not always obtained, and search was made for some factor which would account for the lack of uniformity in the results. Neither the season of the year, the variety, modifications in the nutrient solution, nor the addition of other trace elements appeared to be responsible, and further work is necessary for the elucidation of the problem.

I. INTRODUCTION AND TECHNIQUE

During the course of some solution culture investigations with Chilean nitrate, which were chiefly concerned with its boron and iodine content, attention was drawn to the various trace elements, other than these two which may occur in this material. The consignment received in 1938 by courtesy of the Chilean Nitrate Company contained small traces of chromium, molybdenum, strontium, titanium, vanadium and zinc as was shown by spectrographic analyses carried out by Dr Judd Lewis. To test the effect of these elements on plant growth, lettuces were grown in a modification of van der Crone nutrient solution,* potassium nitrate being replaced by equivalent nitrogen and potassium in sodium nitrate and potassium chloride. The six elements were added singly or in combination in some convenient form, at the rate of 0·1 p.p.m. Boron and iodine equivalent to that present in the Chilean nitrate sample were supplied as boric acid and potassium iodate, the quantities required per litre being 0·0026 g. H_3BO_3 and 0·000524 g. KIO_3 respectively. A similar nutrient solution without the addition of any of the six trace elements served as control, each treatment having five replicates. Analar chemicals were used throughout. The seeds were germinated on filter paper moistened with distilled water, and the seedlings transferred as young as possible to waxed muslin of suitable mesh, stretched over beakers filled with the appropriate solution. Basal nutrients were diluted to 1/5 at this stage. After approximately 1 week the seedlings were removed in pairs to conical flasks of 100 c.c. capacity, containing half-strength basal nutrients, and after a further 2 weeks were finally set up singly in bottles of 400 c.c., and later 600 or 800 c.c. capacity with full nutrients. The elements were supplied at the maximum concentration of 0·1 p.p.m. from the start, and renewals of solution were made fortnightly.

* Van der Crone nutrient solution and modification:

	g.			
KNO_3	1·0	or	$NaNO_3$	0·8408
			KCl	0·7374
$MgSO_4.7H_2O$	0·5			
$CaSO_4.2H_2O$	0·5			
$Ca_3(PO_4)_2$	0·25			
$Fe_3(PO_4)_2.8H_2O$	0·25			
$MnSO_4.4H_2O$	0·001			
H_3BO_3	0·001	or		0·0026
Water	1 l.			

II. COMPARATIVE EFFECT OF Cr, Mo, Sr, Ti, V AND Zn

The effects of molybdenum, strontium, titanium and vanadium were compared in the first experiment (23 July–30 Sept. 1941); molybdenum, chromium, titanium and zinc in the second of the series (25 Aug.–22 Nov. 1941); and finally all six were tested in the third experiment (8 Oct. 1941–2 Feb. 1942). Iodine was supplied to all plants in the first two sets, but in the third, the effect of the other elements was compared in its presence and absence.

The six elements were supplied at the rate of 0·1 p.p.m. in the following forms: chromic acid (H_2CrO_4), sodium molybdate ($Na_2MoO_4.2H_2O$), strontium nitrate ($Sr(NO_3)_2$), titanium trichloride ($TiCl_3$), vanadium oxydichloride ($VOCl_2$) and zinc sulphate ($ZnSO_4.7H_2O$). Tom Thumb was the variety of lettuce grown in the first two sets, but Cheshunt Early Giant was used later during the winter.

(*a*) *With iodine throughout* (*var. Tom Thumb*)

Considerable individual variation occurred among the plants grown in the first experiment in the late summer, and a number died prematurely and had to be discarded. Conditions were more favourable in the autumn and a greater uniformity was obtained

which is reflected in the smaller standard error for the dry-weight figures (Table 1).

TABLE 1. *Comparison between growth of plants treated with various elements. Lettuce, Tom Thumb. Total dry weight per plant* (*g.*) (*mean of* 5)

Treatment (All with iodine)	23 July–30 Sept. 1941 No molybdenum	With molybdenum
Control	1·71*	5·23
Sr	3·00	6·38†
Ti	2·31	6·28
V	4·34†	4·69†
Sr+Ti	4·00†	5·36
Sr+V	5·41	6·38
Ti+V	3·00†	5·85†
Sr+Ti+V	3·02	5·91
Mean	3·35	5·76
S.E. of mean	0·47	
(All with iodine)	25 Aug.–22 Nov. 1941	
Control	1·60	5·10
Cr	3·47	4·93
Ti	3·01	5·24
Zn	2·95	5·95
Cr+Ti	4·44	4·72
Cr+Zn	3·88	5·92
Ti+Zn	4·20	5·76
Cr+Ti+Zn	3·08	6·34
Mean	3·33	5·49
S.E. of mean	0·37	

* Mean of 3. † Mean of 4.

The similarity in the level of the yields from the two experiments, however, indicates that the results from the first set were not vitiated either by the poorer growing conditions or the reduction in the number of replicates.

Visual differences between treatments were sometimes transient, being noticeable either for a short time during the early stages of growth or only when the plants were mature. Lettuces receiving zinc, chromium, titanium + vanadium and strontium + titanium + vanadium, for example, did not maintain in appearance the promise they gave as seedlings, although the set with chromium ultimately gave a significantly higher dry weight than the controls. Vanadium alone or combined with strontium yielded some very good plants, but they did not reach the standard of those supplied with molybdenum which were outstandingly the best. Sets receiving molybdenum alone or with the addition of other elements were conspicuous in a randomized group of plants by their rich green colour and large size. In most cases this improvement in appearance was accompanied by a marked rise in dry weight, and, if the treatments are grouped according as to whether or not molybdenum was supplied, the average dry weight of all plants grown without the element is barely 60% of that where it was included (Table 1). On the basis of yield, some benefit also appears to be derived from the addition of chromium, strontium, titanium or vanadium but the effects are masked in the presence of molybdenum.

(*b*) *With and without iodine* (*var. Cheshunt Early Giant*)

In this experiment, carried out in the winter, iodine was omitted from the solution supplied to half of the plants, to obtain a clearer idea of the effect of the six trace elements themselves and to see if there were any interaction between them and the iodine. Outstanding benefit was again obtained from the addition of molybdenum, both as regards appearance of the plants and their dry weight, though the presence or absence of iodine was immaterial (Table 2). Plants grown with titanium or vanadium gave slightly higher dry weights than the controls, but compared very unfavourably in appearance as well as in yield with those receiving molybdenum. Chromium and strontium were apparently without effect on growth, but zinc was slightly harmful. There was some indication that iodine was exerting an influence on the response of the plants to these three elements, beneficial in the case of titanium and harmful with vanadium and zinc, but further evidence would be required before any specific claim could be made for such an interaction. The appearance of the variously treated plants, in the series receiving iodine, is shown in Pl. 10, fig. 1.

TABLE 2. *Effect of iodine on the response to various elements.* 8 *Oct.* 1941–2 *Feb.* 1942. *Lettuce, Cheshunt Early Giant. Total dry weight per plant* (*g.*) (*mean of* 5)

Treatment	No iodine	With iodine
Control	2·00*	2·81
Cr	2·53	2·61
Mo	6·22	5·56
Sr	2·32†	2·74
Ti	2·32	3·70
V	3·62	2·35
Zn	1·39	0·76
S.E. of mean	0·41	

* Mean of 10 plants. † Mean of 4 plants.

III. EFFECT OF MOLYBDENUM IN THE PRESENCE AND ABSENCE OF BORON

Lettuces grown without molybdenum, however poorly developed, were invariably healthy at the centre of the rosette (Pl. 10, fig. 2). A lack of boron, on the other hand, prevented Tom Thumb plants from developing much beyond the seedling stage. Their central leaves turned brown and leathery, sometimes curling backwards, and the growing point

died as described by McHargue & Calfee (1933), (Pl. 10, fig. 3). This effect was obtained in plants grown during the winter (10 Nov. 1941–23 Mar. 1942) and also in the spring (16 Mar.–29 May 1942). While the addition of iodine (as 0·524 mg. KIO_3 per l.) had little influence on the rate of appearance of boron deficiency symptoms, molybdenum (0·1 p.p.m.) in one instance exerted a delaying action of 2–3 weeks. This was reflected in the additional dry weight laid down in boron deficient plants where molybdenum was supplied (Table 3). With both

TABLE 3. *Effect of boron on the response to iodine and molybdenum. Lettuce. Total dry weight per plant (g.) (mean of 5)*

	10 Nov. 1941–23 Mar. 1942	
Treatment	Tom Thumb	Cheshunt Early Ball
No boron:		
Control	0·10	Not grown
Iodine	0·26	
Molybdenum	1·44	
With boron:		
Control	0·54	1·62
Iodine	0·78	2·00
Molybdenum	4·13	7·16
I+Mo	4·77	6·83
S.E. of mean	0·25	0·34

Tom Thumb and Early Ball the responses to iodine and molybdenum in the presence of boron confirmed the results already described, viz. the failure of iodine to exert any appreciable influence on growth, and the capacity of molybdenum alone or in combination to improve both colour and size. The beneficial effect of molybdenum was better defined in Early Ball than in Tom Thumb during the winter, as would be expected from the fact that the former is a winter variety. The question of the possible importance of the amount of boron supplied is discussed in a later section.

IV. RESPONSE TO A DEFICIENCY OF MOLYBDENUM

Evidence of the beneficial action of molybdenum to which attention had been already drawn by Brenchley & Warington (1942) had now been obtained on five successive occasions, and was so well defined as to suggest that molybdenum was essential for the normal development of lettuce. Arnon (1938) found that it was among a group of seven elements, which together had a favourable action on the colour and size of lettuce grown in nutrient solution, while Arnon & Stout (1939) have claimed that it is essential for the growth of tomato. The degree of response in the experiments now under consideration was, however, variable. In 1941 and on several occasions in 1942 and again in 1944 the effect was well defined, the plants without molybdenum being small and pale with a loose, open habit. On approaching maturity they developed a frill of withered leaves between the still healthy inner and outer whorls. This withering first showed itself as patches of grey-blue crinkled tissue chiefly at the apices of the medium aged leaves (Pl. 10, fig. 4). Later these patches coalesced, turned yellow and papery, and if several adjacent leaves were similarly affected, a frilled appearance resulted (Pl. 10, fig. 2). On some occasions in 1942 and 1943, however, the plants without molybdenum were quite healthy and normal, though there was a definite improvement in colour and size where the element was supplied. In yet other cases, chiefly in 1943, no deficiency or beneficial symptoms appeared at all, the treated and untreated plants growing equally vigorously, levelling up to the better rather than down to the poorer standard. Experiments were accordingly laid down to see if some explanation could be found for these seemingly conflicting results and these are described below.

V. ENVIRONMENTAL CONDITIONS IN RELATION TO THE RESPONSE TO MOLYBDENUM

(*a*) *Various concentrations of molybdenum*

Hitherto molybdenum had been supplied at the rate of 0·1 p.p.m. only. A range of concentrations, each ten times stronger than the next (extending from 0·001 to 100 p.p.m.), was now tried, with a no molybdenum control, at three different seasons in the year during 1942–3, viz. 14 Feb.–11 May, 6 May–29 June and 21 Sept.–22 Feb., Tom Thumb, Trocadero, Cheshunt Early Ball and Cheshunt Early Giant being the varieties selected. The modification of Crone's solution was used in the first of these experiments, but the more usual formula was employed on the two later occasions. The dry-weight figures show that very satisfactory growth was made, and with the exception of the plants receiving the highest concentration of molybdenum, development was uniformly good throughout (Table 4).

Toxic effects were universally shown with 100 p.p.m. Mo, though their severity depended to some extent on the variety and the time of year at which the plants were grown. The results here described, therefore, are not necessarily of general application. The symptoms showed first as a yellow-brown discoloration of the roots, but growth of the shoot was also severely checked and the leaves turned a golden yellow. Tom Thumb was particularly sensitive and showed damage even in the cotyledon stage with 100 p.p.m. Mo, whereas Early Ball seedlings grown alongside remained healthy for a further 5 days, though both varieties died

eventually. Slight root discoloration appeared in Tom Thumb with even 10 p.p.m., but not in the other varieties. Early Giant was also more susceptible to injury than Early Ball. Trocadero, on the other hand, was very resistant and showed considerable capacity for recovery, and though damaged by 100 p.p.m. it eventually reached a dry weight of almost 60% of the controls, whereas Tom Thumb under similar conditions barely attained 18%. Recovery to a limited extent took place on one occasion in the roots of badly poisoned Tom Thumb plants, but the new growth was quite abnormal. The early formed fine, discoloured roots dried up or rotted off at the point of attachment and unusually short, thick, white roots developed in their place (Pl. 10, fig. 5). Rotting at the junction of the root and shoot had previously been noticed in barley grown in soil treated with too high a concentration of molybdenum (Warington, 1937). With 10–0·001 p.p.m. Mo, therefore, that seasonal conditions might be influencing the response to molybdenum and account for the apparently conflicting results just described. Tom Thumb lettuce was accordingly sown at weekly intervals for the year May 1942–April 1943 and fortnightly from June 1943 to May 1944. Cheshunt Early Giant was also sown fortnightly from November 1943 to May 1944, care being taken to use the same batch of seed throughout each series. Five plants from each sowing were given 0·1 p.p.m. Mo (as sodium molybdate), while five received no molybdenum, the normal Crone nutrient solution containing KNO_3 being used. As would be expected, considerable fluctuations occurred in yield throughout the year and indications of their range will be found in Table 5 and Text-fig. 1. Most of the lower dry weights for both the 1942–3 and 1943–4 series with the variety Tom Thumb were obtained from September, October or November sowings, while

TABLE 4. *Effect of various concentrations of molybdenum on growth of lettuce. Total dry weight per plant* (g.) (*mean of* 5)

	14 Feb.–11 May 1942		6 May–29 June 1942		21 Sept. 1942–22 Feb. 1943	
Treatment	Tom Thumb	Early Ball	Tom Thumb	Trocadero	Early Giant	Early Ball
Control, no Mo	9·03	8·40	8·30	20·87	8·77	7·70
Mo 0·001 p.p.m.	8·92	8·38	7·99	17·87	9·18	8·10*
Mo 0·01 p.p.m.	7·26	8·08	7·37	19·28	8·80	8·42*
Mo 0·1 p.p.m.	8·46	7·63	7·71	20·11	8·82	8·01
Mo 1 p.p.m.	7·88	7·34	8·61	19·16	7·90	8·75*
Mo 10 p.p.m.	7·81	7·91	7·92	20·56	10·65*	8·40
Mo 100 p.p.m.	0·10	0·21	1·49	12·41	0·11	0·09*
S.E. of mean (omitting highest concentration)	0·71	0·37	0·46	0·79	0·66	0·61

* Mean of 4.

growth was remarkably uniform and there was no indication of an optimum concentration. Too much weight, however, must not be attached to this conclusion for the controls also were healthy as the high dry-weight figures show. Some factor was evidently preventing the development of the symptoms that had previously occurred in the absence of molybdenum, but so far it remains undetermined. The change in the results cannot, however, be attributed to the introduction of molybdenum through the use of a different batch of salts, as identical stocks of chemicals were used in this and in the earlier experiments when the response to molybdenum was so striking.

(*b*) *Seasonal conditions*

Arnon (1938) found that lettuce showed more benefit from the addition of trace elements (including Mo) in summer than in autumn, and it is common knowledge that the boron requirement of plants may vary with the time of year. It seemed possible, the best yields came from seed sown in April and May for the 1942–3 set and in June and July for the 1943–4 experiment. These seasonal fluctuations were independent of treatment with molybdenum and the curves for the yields of plants grown with and without this element followed one another very closely in direction. Less seasonal variation was shown by the variety Early Giant, as it was grown for a period of only 6 months almost entirely under favourable conditions. Response to molybdenum was variable. In some cases striking improvement in both colour and dry weight occurred when it was supplied, in others the benefit showed in dry weight only, but there were only two instances of any improvement in colour without an accompanying increase in dry weight. On a few occasions the plants without molybdenum gave the higher yield, but taken on the whole there was a slight significant balance in favour of the addition of molybdenum in all three series (Table 5).

Attempts to correlate these responses to molyb-

denum with season were disappointing as the peak periods were not the same in the 2 years (Text-fig. 2). The visual benefit from molybdenum in the Tom Thumb plants was most marked from April to June in the 1942–3 series, whereas in the following year it was greatest from Nov. to Mar. The results for Early Giant are not strictly comparable with those for Tom Thumb, as the former variety is unsuitable for summer growing under glass and any response was necessarily restricted to the winter months. On a few occasions the addition of molybdenum seemed to retard bolting, the control plants starting to run up several days earlier than the rest. At other times no such differences were noticeable, though as the plants were sometimes harvested before this stage was reached, it was not possible to estimate the frequency of the occurrence.

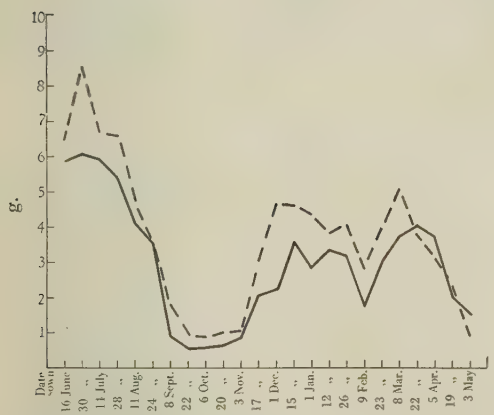
responses to molybdenum that had occurred, lettuces were grown with and without molybdenum, in all the forms of the Crone nutrient solution that had been used, thus making direct comparison between them under identical seasonal conditions. The Crone solutions were as follows:

I. Usual form with KNO_3 (see footnote, p. 249).
II. $NaNO_3 + KCl$ in place of KNO_3 (see footnote, p. 249).
III. As (II) but made up from a different batch of chemicals, with negative for Mo on spectrographic analysis.
IV. Chilean nitrate + KCl in place of KNO_3.

In the first instance, solutions (I) and (II) only were compared, a single level of molybdenum (0·1 p.p.m. Mo) and boric acid (1 mg./l.) being used.

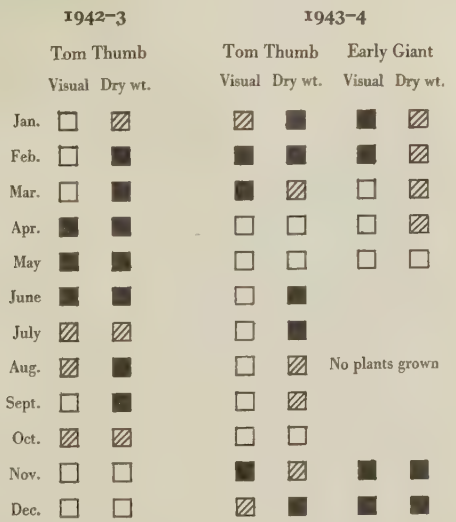
TABLE 5. *Response to molybdenum during two years of regular periodic sowings. Total dry weight per plant (g.)*

	No molybdenum		With molybdenum		S.E. of average
Tom Thumb	Mean	Range of mean	Mean	Range of mean	difference ± Mo
All plants, 52 sowings, 1942–3	5·16	(2·02–11·22)	5·65	(2·13–14·16)	0·16
All plants, 24 sowings, 1943–4	2·98	(0·56– 6·08)	3·70	(0·87– 8·52)	0·16
Cheshunt Early Giant					
All plants, 14 sowings, 1943–4	3·98	(1·89– 6·66)	5·10	(2·09– 7·41)	0·29



Text-fig. 1. Tom Thumb lettuce. Dry weight per plant (mean of 5). Fortnightly sowings, 1943–4.

—— No molybdenum. - - - - 0·1 p.p.m. Mo.



Text-fig. 2. Comparison of visual and dry-weight response to molybdenum. □ No benefit from molybdenum. ▨ Colour benefit and/or dry-weight increase shown in one of the sowings per month. ■ Marked colour benefit and/or dry-weight increase shown in two or more of the sowings per month. Weekly sowings, 1942–3. Fortnightly sowings, 1943–4.

(*c*) *Modifications in the nutrient solution*

As mentioned in § I, slight modifications in the composition of the usual Crone nutrient solution had been made in the earlier experiments to afford the desired comparison with a given sample of Chilean nitrate. In order to make sure that such alterations were not responsible for the varying

In a later experiment all four solutions were tested simultaneously, molybdenum and boric acid being included at two rates, viz. 0·1 and 1·0 p.p.m. Mo and 1·0 (low) and 2·6 mg. (high) H_3BO_3 per l. respectively. No boric acid was added to (IV) as the Chilean nitrate already contained boron at the higher level. It was, therefore, not possible to test the effect of the smaller quantity on plants receiving their nitrogen in this form. The dry-weight figures are given in Table 6.

Good growth was made irrespective of the form of the nutrient solution, and although there were small significant differences between some of the treatments, taken on an average there was no response to molybdenum, nor was the quantity of boron supplied important. An explanation for the varied response to molybdenum was, therefore, not forthcoming along these lines.

There remained the possibility that for the effective action of molybdenum, the presence of some trace element or elements, other than the six already tried, might be necessary. Such an explanation would, however, presuppose the addition of the element as an impurity in some, but not all solutions used on previous occasions. This was unlikely, since the same batch of chemicals had been used over most, if not all, of the period during which the response to molybdenum had varied. The effect of a solution containing all the elements in Hoagland's A–Z mixture, with the exception of molybdenum (Hoagland & Snyder, 1933) was, however, tried on a number of plants, including lettuce, with and without the addition of 0·1 p.p.m. Mo, but there was no indication of any benefit either from the addition of the composite mixture, the molybdenum or both solutions together.

Table 6. *Comparison between the effect of different forms of nutrient solution on the response to molybdenum. Dry weight per plant* (g.) (*mean of* 5). *Cheshunt Early Giant*, 1943

	5 Jan.– 6 April		(8 Feb.–7 May)					
	Low B		No Mo		0·1 p.p.m. Mo		1·0 p.p.m. Mo	
Form of N and K in Crone nutrient solution	No Mo	0·1 p.p.m. Mo	Low B	High B	Low B	High B	Low B	High B
(I) KNO_3	6·63	5·94	10·16	9·54	11·64	10·20	9·74	10·59
(II) $NaNO_3$ + KCl	7·11	5·78	10·61	10·03	11·36	10·67	9·67	10·17
(III) $NaNO_3$ + KCl	—	—	—	10·72	—	9·71	—	10·62
(IV) Chilean nitrate + KCl	—	—	—	9·64	—	9·79	—	9·89
	S.E. of mean 0·31		S.E. of mean 0·51					

VI. General conclusions

In reviewing the results of all these experiments, one is forced to the conclusion that in spite of the occasions on which no response was obtained, molybdenum plays an important part in maintaining the normal health of lettuce. Such striking proof was obtained so frequently in support of this view, that the negative results can only be regarded as exceptions, and it is to be hoped that on further investigation some satisfactory explanation of the matter will ultimately be found.

The author's thanks are due to Dr W. E. Brenchley for helpful criticism of the manuscript, to Mr V. Stansfield for the photographs, and to Miss E. Rabl for assistance in growing and harvesting the plants.

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EXPLANATION OF PLATE 10

Fig. 1. Early Giant lettuce. Treatments left to right: Control, Mo, Ti, Cr, Sr, V, Zn, all 0·1 p.p.m.

Fig. 2. Early Giant lettuce grown without molybdenum, showing healthy centre and withering of medium aged leaves.

Fig. 3. Tom Thumb lettuce grown without boron, showing death of growing point at centre.

Fig. 4. Early Giant lettuce grown without molybdenum. Leaves showing withered apices and margins.

Fig. 5. Tom Thumb lettuce poisoned by excess molybdenum, showing the two types of abnormal roots.

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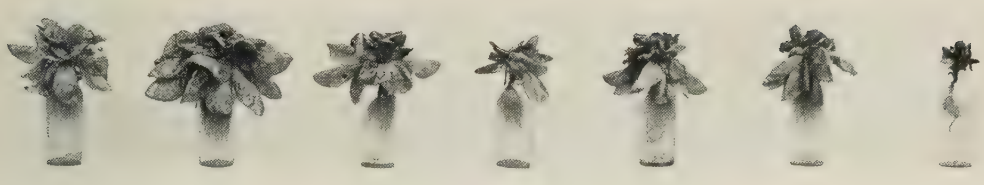


Fig. 1

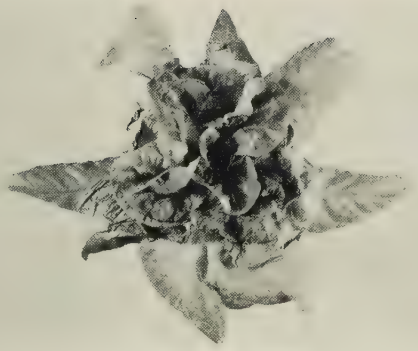


Fig. 2



Fig. 3

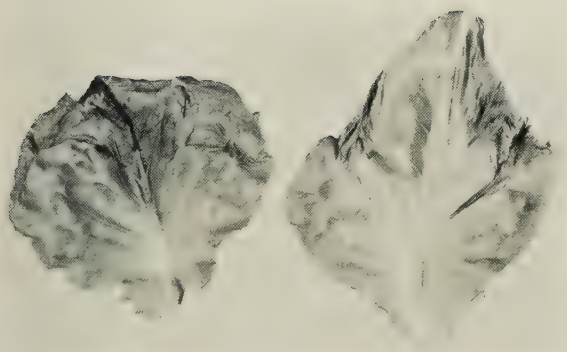


Fig. 4



Fig. 5

Observations on the effects of browning (*Polyspora Lini* Laff.) of flax on seed production

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An account is given of observations made on the effects on seed production of the incidence of the browning phase of the disease of flax caused by *Polyspora Lini* Laff. The work was carried out during three seasons.

Disinfection of seeds before sowing exercises an effect on the average weight of the well-developed seeds harvested, and the results obtained show that the intensity of the attack of browning in crops is inversely correlated with the weight of 1000 well-developed seeds. When poorly developed seeds are separated from the remainder at harvest, evidence obtained in one year suggests that the percentage number of such seeds may be directly related to the incidence of browning in the crop. It was found in each of two years that more of the poorly-developed than of the well-developed seeds were contaminated with *P. Lini*. Evidence available for one year suggests that the viability of well-developed seeds is not related to the incidence of browning.

It was observed by Lafferty (1921) and Henry (1928) that a crop of flax affected with stem-break and browning (*Polyspora Lini* Laff.) produces a seed yield which is lower in quantity and quality than that harvested from a healthy crop. Further work was carried out in Russia by Kazina (1935) using seeds artificially contaminated with spores of *P. lini* to varying extents. She found that if less than 8·5% of the seeds sown were contaminated the quantity and quality of the seeds produced by the crop were not appreciably affected, but if more than 8·5% of the seeds were contaminated then the yield was reduced in proportion to the increasing contamination of the seed sown. When only contaminated seeds were sown Kazina found that the yield was reduced by about 30%. Recent observations made in Great Britain (Moore, 1943) indicated that the disease reduces the viability as well as the yield of the seed, whereas Kazina (1935) reported that the percentage of viable seeds among those harvested was not related to the intensity of crop attacks.

Since 1939 extensive field trials designed to test the efficacy of various seed disinfectants for the control of stem-break and browning have been carried out in Northern Ireland (Muskett & Colhoun, 1942, 1943, 1944). In this work seed naturally contaminated with *P. Lini* was subjected to treatments of varying efficiency, and the intensity of each of the three phases of the disease developing in the crops from treated seed was compared with that in crops from untreated seed. During the investigation careful observations were made on the crops which were grown from the same seed sample but which, because of the different treatments applied, showed each phase of the disease to varying extents. Material from these crops was utilized for studying the effects of the disease on seed production.

Observations made in 1941

Seed bolls were collected at harvest from crops in each of forty-four plots sown with the variety Blue Star, those collected from each plot being kept separate. Four plots had been sown with untreated seed, while an equal number of plots was sown with each lot of treated seed. The treatments employed are specified in Table 1, in which the percentage number of seeds contaminated with *P. Lini* in each case is also given. The values for the contamination of treated and untreated seed were determined by the method described by Muskett & Colhoun (1942). Observations were made two weeks before pulling time on the incidence of the browning phase in each plot, an assessment mark of 10 being given to the most severely attacked, while the marking of the others varied between 10 and 0 according to the amount of infection present. The average value calculated for the four plots sown with each lot of seed is stated in Table 1. The seed bolls collected from each crop were crushed and sieved to extract the seeds which were then exposed to a vertical blast of air in an aspirator of the type described and illustrated by Mercer (1937), so that all light seeds and pieces of chaff were removed. The weight of 1000 seeds (afterwards referred to as the *M* weight) from each lot was then determined, the results obtained being presented in Table 1.

Analysis of variance was applied to the data obtained for the *M* weight; the value of *Z* obtained for treatment (+0·44438) exceeds the appropriate 5% probability value (0·3891) and hence is highly significant. This result indicates that disinfection of the seed before sowing exercises a real effect on the average weight of the winnowed seeds produced from the crop.

The correlation between the intensity of attack by the browning phase and the *M* weight of the seeds has been calculated, using the values obtained for each of the forty-four plots. The result obtained was as follows: $r = -0{\cdot}7829$. This correlation is highly significant, since the value obtained exceeds the appropriate 0·1% probability value (0·4797) and indicates that the intensity of the attack by the browning phase is inversely correlated with the *M* weight of the winnowed seeds.

Observations made in 1942

Seed bolls were collected from each of the twelve plots constituting a block in one of the field trials carefully separated by hand from the chaff so that all the seeds produced in the bolls were retained irrespective of whether or not they had developed normally. By exposing all the seeds to a blast of air in the aspirator under standard conditions, the light seeds were separated from those which had undergone fairly normal development. The weights of the seeds in the heavy and light grades were then determined for each sample, and from this data the percentage weight in the light grade was calculated. The *M* weight was determined for the seeds in each of the light and heavy grades by weighing 1000 seeds from each, and using this data the percentage number of seeds in the light grade was calculated for each

Table 1. *The effect of browning on seed production*, 1941

Treatment	Rate of application per 500 g.	Laboratory test (500 seeds examined) % contaminated seeds	Field test Browning phase Maximum attack = 10	Weight of 1000 seeds harvested* (g.)
Untreated	—	50·4	10	3·592
Agrosan G	6·0 g.	37·8	6	**3·916**
Ceresan (1875 A)	6·0 g.	23·6	7	**3·871**
Ceresan (1875 A) Fixed with separated milk	3·0 g. + 40 ml.	15·8	4	**4·078**
Nomersan	1·5 g.	35·6	8	3·813
Nomersan	3·35 g.	28·2	7	**3·857**
Nomersan	6·0 g.	19·6	7	**3·906**
Nomersan Fixed with separated milk	3·0 g. + 40 ml.	27·6	8	3·754
Ceresan (U 564) 4%	40 ml.	31·4	7	**3·891**
Ceresan (U 564) 8%	30 ml.	29·4	7	**3·876**
Ceresan (U 564) 8%	40 ml.	20·4	4	**4·046**

* Mean values for treatments showing significantly higher values than that for the control are given in heavy type.

made during the year with the variety Liral Crown. One plot had been sown with untreated seed, and each of the remaining plots with seeds which had undergone one of the treatments mentioned in Table 2. The percentage number of seeds contaminated with *P. Lini* in each lot of seed used for sowing and the value for the incidence of the browning phase in each plot at pulling time were determined and are given in Table 2. The bolls collected from each plot were crushed and sieved as in 1941, but in this year, although a controlled blast of air was again utilized for the separation of well-developed seeds from the chaff and light seeds, the latter were sample. Employing the Ulster method (Muskett & Malone, 1941), 500 seeds from each of the light and heavy grades were examined for contamination with *P. Lini*. The results are presented in Table 2.

The values for the incidence of browning in the crop were correlated with those for the percentage number of seeds contaminated with *P. Lini* among those sown, as well as with the percentage weight and number of light seeds harvested, the *M* weight of light and heavy seeds and the percentage number of light and heavy seeds contaminated with *P. Lini*. The results obtained are as follows:

Browning in crop v. percentage number of seeds sown contaminated with *P. Lini*	$r = +0{\cdot}7789$
,, ,, v. percentage weight of light seeds	$r = +0{\cdot}5183$
,, ,, v. percentage number of light seeds	$r = +0{\cdot}7409$
,, ,, v. *M* weight of light seeds	$r = -0{\cdot}5476$
,, ,, v. *M* weight of heavy seeds	$r = -0{\cdot}7851$
,, ,, v. percentage number of light seeds contaminated with *P. Lini*	$r = +0{\cdot}3696$
,, ,, v. percentage number of heavy seeds contaminated with *P. Lini*	$r = +0{\cdot}6768$

(Probability values: $P = 0{\cdot}05$, 0·5760; $P = 0{\cdot}01$, 0·7079 when $n = 10$.)

TABLE 2. *Effect of browning on seed production, 1942*

Treatment	Rate of application per 500 g.	Laboratory test (500 seeds examined) % number of seeds contaminated with *P. Lini* among those sown	Incidence of browning phase on crop at pulling time Maximum attack = 10	% weight light seeds	% number light seeds	*M* weight light seeds (g.)	% number of light seeds contaminated with *P. Lini*	*M* weight heavy seeds (g.)	% number of heavy seeds contaminated with *P. Lini*
Untreated	—	22·4	10	6·0	22·5	0·793	46·6	3·624	15·6
Nomersan	3·35 g.	9·8	10	4·7	19·3	0·770	49·8	3·721	22·6
Ceresan (1875 A) Fixed with water	3·35 g. 30 ml.	8·1	2	4·4	17·3	0·846	48·4	3·841	12·6
Ceresan (1875 A) Fixed with water	3·35 g. 40 ml.	8·4	1·5	5·8	17·7	1·113	44·0	3·891	6·4
Ceresan (experimental) Fixed with water	3·35 g. 30 ml.	3·8	1	2·8	12·6	0·822	34·2	4·122	5·6
Ceresan (experimental) Fixed with water	3·35 g. 40 ml.	3·2	0·5	3·6	13·1	0·981	44·6	3·911	9·0
Ceresan (U 564) 6 %	40 ml.	8·4	1	5·1	18·4	0·942	55·6	3·945	20·0
Ceresan (U 564) 8 %	30 ml.	8·4	1	3·9	14·8	0·886	43·4	3·872	11·8
Ceresan (U 564) 8 %	40 ml.	6·4	1	5·0	17·7	0·974	36·6	3·956	4·8
Ceresan (U 564/2/B 1) 6 %	40 ml.	1·8	1	3·0	11·9	0·884	16·6	3·887	5·8
Ceresan (U 564/2/B 1) 8 %	30 ml.	2·8	0·5	3·0	13·7	0·816	38·8	4·164	6·4
Ceresan (U 564/2/B 1) 8 %	40 ml.	1·8	0·5	3·7	13·5	0·982	28·6	4·023	5·6

TABLE 3. *Effect of browning on seed production, 1944*

Treatment	Rate of application per 500 g.	Laboratory test (500 seeds examined) % number of seeds contaminated with *P. Lini* among those sown	Incidence of browning phase on crop at pulling time Maximum attack = 10	% weight light seeds	% number light seeds	*M* weight light seeds (g.)	% number of light seeds contaminated with *P. Lini*	*M* weight heavy seeds (g.)	% number of heavy seeds contaminated with *P. Lini*
Untreated	—	14·6	10	3·36	10·4	1·410	42·0	4·235	22·8
Nomersan	3·35 g.	9·2	6	1·13	5·3	1·008	8·8	4·655	4·4
Ceresan (1875 A) Fixed with water	3·35 g. 30 ml.	7·4	3	0·90	3·8	1·128	18·6	4·750	6·4
Ceresan (U 564) 8 %	30 ml.	4·2	0	1·08	4·3	1·200	9·3	4·720	3·2
Ceresan (U 564/2/B 1) 8 %	30 ml.	5·0	2	0·79	3·5	1·040	6·0	4·749	3·6
Ceresan (U 564/2/B 1) 8 %	40 ml.	2·2	2	0·63	2·2	1·400	11·0	4·838	1·2

Four of the correlation coefficients obtained exceed the appropriate 5% point, and these being regarded as significant are given in heavy type. In considering these correlations it must be emphasized that only twelve pairs of values entered into the calculation. The coefficient of correlation between the incidence of the browning phase in the crop and the *M* weight of heavy seeds is again negative and exceeds the appropriate 1% probability value; this is in agreement with the finding in 1941. The results obtained suggest that the percentage number of seeds sown contaminated with *P. Lini* is directly correlated with the incidence of the browning phase in the crop, and also that the heavier the attack of browning the higher will be the percentage number of light seeds and the higher will be the percentage number of heavy seeds contaminated with *P. Lini*.

It should be noted from Table 2 that the number of seeds contaminated with *P. Lini* was in each case always much higher in the light than in the heavy seeds.

Observations made in 1944

Seed bolls were collected from six plots comprising a block in a field trial made with the variety Stormont Gossamer. One plot had been sown with untreated seed, while each of the other plots was sown with seed from the same sample which had been treated with one of the fungicides listed in Table 3. As in 1942 the value for the percentage number of contaminated seeds in each lot sown, as well as that for the incidence of the browning phase in each plot, was determined and is presented in Table 3. Following the same procedure as described in the case of the 1942 crops data regarding the percentage weight and percentage number of light seeds, the *M* weight of light and heavy seeds as well as the percentage contamination with *P. Lini* of light and heavy seeds were collected for each sample, the results being presented in Table 3. It should be noted that in general the results obtained in 1944 are in agreement with those obtained in 1941. Since values are only available for six plots the correlation coefficients between pairs of values have not been calculated.

Germination tests showed that in so far as the well-developed seeds are concerned there were no differences in the percentage number of viable seeds collected from each plot, since in each case 99% of the seeds in the heavy grade germinated inside 5 days. The viability of the seeds in the light grade was not tested.

At pulling time a number of plants were selected showing well-developed symptoms of browning on the stems, and at the same time plants were selected which after careful examination were found to be healthy. All the bolls, sixty-three in number, were detached from sixteen infected plants, the seeds from each boll kept separate and examined by the Ulster method for contamination with *P. Lini*. It was found that of the 591 seeds examined 115 were contaminated with *P. Lini* and 526 were classed as heavy seeds. When an equal number of bolls were detached from eleven healthy plants and the seeds treated in the same way as those from bolls on diseased plants, it was found that of the 606 seeds examined one was infected with *P. Lini* while 562 were classed as heavy seeds. The results obtained showed that the seeds in some bolls on an infected plant may be free from contamination, while in other bolls on the same plant all, or only a proportion, of the seeds may be contaminated with the fungus. The almost complete absence of infection of seeds on the healthy plants should be noted.

Discussion

It has been shown by Muskett & Colhoun (1942) that when the incidence of browning in crops is considered shortly after its first appearance, then it is directly correlated with the number of seeds contaminated with *P. Lini* among those sown. Since browning spreads rapidly it might be expected that with the passage of time from its first appearance, this relationship will become less marked. The results presented in this paper, however, show that it was possible to demonstrate in one year that the incidence of browning at pulling time was still correlated with the percentage number of contaminated seeds sown.

In 1941, the results obtained show that disinfection of the seed before sowing exercises an effect on the *M* weight of the heavy or winnowed seeds harvested. Both in this year and in 1942 it was found that the incidence of browning in crops was inversely correlated with the *M* weight of the heavy seeds. This result indicates that as the intensity of the browning phase becomes heavier the *M* weight of the winnowed seeds becomes lower. In view of these results it may be suggested that, on the average, the developing seeds in a crop grown from heavily contaminated seeds are attacked at an earlier date than those in the case of comparable crops produced from less heavily contaminated or disinfected seeds. This suggestion is supported by the observation made during a number of seasons that browning spreads more slowly in crops from disinfected than from undisinfected seeds.

In 1942, the only year for which the values are available, no significant correlation was established between the incidence of browning in crops and either the *M* weight of the light seeds or the percentage weight of light seeds harvested. A direct and significant correlation was found to exist between the incidence of browning and the percentage number of light seeds. In considering these results it should be pointed out that in most of the samples examined a very large number of the light seeds were un-

developed and extremely light in weight. The results obtained show that in 1942 the incidence of browning was directly related to the number of heavy seeds contaminated with *P. Lini* at harvest; this is in agreement with the findings of Kazina (1935). It must be stated, however, that unpublished results obtained over a number of years in Northern Ireland demonstrate that this relationship does not exist in every season.

It is of interest to note that in each of the two years for which results are available a higher percentage of the light seeds than of the heavy seeds was contaminated with *P. Lini*. It was always possible to find light seeds in a sample which were not contaminated, and in certain samples the percentage number of the contaminated light seeds was low.

The effect of browning on the germination of the seeds was studied in one year only, and values were obtained for the viability of *heavy* seeds from crops affected with browning to varying degrees. However, no tests of the viability of the *light* seeds were made. The results obtained show that the germination of the seeds examined was not influenced by their contamination with *P. Lini* or by the incidence of browning in the crops from which they were harvested. This conclusion agrees with the finding of Kazina (1935) that the intensity of browning in the crop did not reduce the percentage of the viable seeds harvested.

The writer wishes to express his thanks to Prof. A. E. Muskett for his interest and criticism and to the Agricultural Research Institute, Hillsborough, for the provision of field facilities. Acknowledgement is made to the Flax Development Committee for its generosity in continuing to provide a grant-in-aid to the Plant Disease Division in support of the investigation of diseases of flax.

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The relation between the contamination of flax seed with *Polyspora Lini* Laff. and *Colletotrichum linicola* Pethybr. & Laff. and the incidence of disease in the crop

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Using naturally contaminated seed it is shown that the incidence of the seedling, stem-break and browning phases of attack by *Polyspora Lini* Laff. in flax crops is related to the contamination of the seed with the parasite, the relationship being closer at low or moderate than at high levels of seed contamination. Heavy attacks of the disease did not occur when the number of contaminated seeds was less than 5%. A similar relationship was found to exist between the contamination of seed with *Colletotrichum linicola* Pethybr. & Laff. and the incidence of seedling blight in the crops.

Since difficulty was experienced in the elimination of *Polyspora Lini* Laff. from contaminated flax seed by seed disinfection (Muskett & Colhoun, 1943), it appeared desirable to obtain some information regarding the effect of sowing seed contaminated to varying extents with this parasite on the incidence of the disease in the crop. Little work of this nature has been reported in the literature, but Kazina (1935), working in Russia, studied the problem, employing artificially contaminated seed. She demonstrated that the percentage number of plants exhibiting the stem-break or browning phase of the disease was related to the number of contaminated seeds among those sown. The data she presented shows that when 3·5% of the seeds were contaminated as many as 17·3% of the stems in the crop exhibited browning before harvest. In the case of the stem-break phase she found that one-fifth of the plants were diseased in a crop grown from seeds all of which were contaminated. The values Kazina obtained for yield showed that contamination of the seed up to 8·5% produced little effect on the yield of either straw or seed; values for the yield of fibre were not given.

When it was decided to study this problem it was considered advisable to use only seeds which were naturally contaminated and to include *Colletotrichum linicola* Pethybr. & Laff. in the scope of the investigation, since an effective means of controlling this parasite had not been found by 1940 when the work was commenced.

Observations made in 1940

Seed samples from crops of a number of varieties grown in 1939 were selected on the basis of the contamination of the seed as determined by the examination of 500 seeds from each sample by the Ulster method (Muskett & Malone, 1941). Thirty samples were chosen to provide a range of contamination with *P. Lini* from 0 to 35·2% and seventeen samples to give a range of contamination with *C. linicola* from 0 to 46·6%. These samples were sown on 22 May in plots measuring 2 × 2 yd., the seed being sown in drills 6 in. apart at the rate of 80 lb./statute acre. Each plot was surrounded by a path 2 yd. wide. Observations were made on the intensity of attack by *P. Lini* and *C. linicola* in the crops on 14 June, an assessment figure of 10 being given to the most heavily infected plots and the marking of the others varying between 10 and 0 according to the amount of infection present. In the plots sown with seed contaminated with *P. Lini*, observations on the stem-break phase were made on 18 July and on the browning phase on 1, 9 and 20 Aug., the same method of assessment of the severity of the disease being employed as in the case of the seedling phase. The results obtained from these observations together with the values for the contamination of the seed samples sown are given in Table 1 for *P. Lini* and in Table 2 for *C. linicola*. The crops were pulled on 22 Aug.

When seed contaminated with *P. Lini* was sown a heavy attack of the disease did not result when the contamination of the seed was lower than 5%. In general the incidence of the disease in the crop was related to the contamination of the seed, but the relationship was closer at low or moderate than at high levels of seed contamination. Crops which remained healthy until pulling time were produced by three seed samples on which the parasite was found to be absent when examined by the Ulster method. However, two other seed samples, also shown to be free from contamination with *P. Lini*, produced crops which although healthy until a few days before pulling time did eventually show a slight degree of infection. This appearance of the disease on mature plants developing from uncontaminated seeds can be attributed to spores being carried to them from diseased plants in adjacent plots.

The results obtained with *C. linicola* show that the

TABLE 1. *Relationship between contamination of seed with* Polyspora Lini *and the incidence of disease in the crop*, 1940

Variety	Laboratory test: Seeds contaminated with *P. Lini* %	Field test: Measure of crop attack by *P. Lini* (maximum attack = 10) — Seedling phase	Stem-break	Browning: 1. vii. 40	9. vii. 40	20. vii. 40	Mean of 3 observations
Stormont Cirrus	0·2	0	0	1	1	1	1·0
,, ,,	0·8	6	0	1	3	3	2·3
,, ,,	1·4	0	0	0	0·5	1	0·5
,, ,,	1·6	3	3	0	0·5	2·5	1·0
,, ,,	2·2	0	0	2	0·5	1·5	1·3
,, ,,	3·6	3	3	2	1·5	2·5	2·0
,, ,,	5·6	6	6	9	5	6	6·7
,, ,,	7·8	10	10	10	10	10	10·0
,, ,,	10·4	10	6	7	7	9	7·7
,, ,,	16·2	10	10	9	9	8	8·7
,, ,,	31·2	10	10	6	9	10	8·3
,, ,,	35·2	10	10	10	10	10	10·0
Liral Dominion	0	0	0	0	0	0	0
,, ,,	0·6	3	3	0	0·5	1·5	0·7
,, ,,	2·0	3	3	2	2·5	5·0	3·2
Liral Monarch	0	0	0	0	0·5	1·0	0·5
,, ,,	0	0	0	0	0·5	1·0	0·5
,, ,,	0·2	0	0	0	0·5	0·5	0·3
,, ,,	3·6	3	6	1	0	1	0·7
Liral Crown	0	0	0	0	0	0	0
,, ,,	0·2	0	0	0	0	0	0
,, ,,	1·3	0	0	0	1	1	0·7
,, ,,	7·0	6	6	2	5	5	4·0
Liral Prince	0·6	0	0	0	0·5	0·5	0·3
Stormont Gossamer	0·1	0	0	0	0	1	0·3
Blue Star	23·7	10	10	4	7·5	7·5	6·3
Unknown	0	0	0	0	0	0	0
,,	0·2	0	0	0	0·5	0·5	0·3
,,	0·4	0	0	0	0·5	1·0	0·5
,,	0·4	0	0	0	0·5	0·5	0·3

Table 2. *Relationship between contamination of seed with* Colletotrichum linicola *and the incidence of disease in the crop*, 1940

Variety	Laboratory test: % seeds contaminated with *C. linicola*	Field test: Measure of crop attack by *C. linicola* (maximum attack = 10) Seedling phase
Stormont Cirrus	0	0
,, ,,	0	0
,, ,,	8·4	3
Liral Dominion	0·4	0
,, ,,	13·2	10
,, ,,	36·6	10
Liral Monarch	0·4	0
,, ,,	3·2	6
,, ,,	9·0	6
,, ,,	32·4	10
Liral Crown	9·6	6
,, ,,	10·2	6
,, ,,	21·2	10
,, ,,	27·0	10
Stormont Gossamer	35·7	10
Unknown	0	0
,,	46·6	10

incidence of the disease in the seedling phase is related to the percentage number of contaminated seeds among those sown. As was the case with *P. Lini* this relationship is not so obvious at the higher ranges of contamination of the seed.

Observations made in 1942

Seed samples exhibiting varying degrees of contamination with either *P. Lini* or *C. linicola* were prepared from a sample known to be heavily contaminated with one of the parasites. The samples were prepared by adding varying quantities of uncontaminated seed of the same variety, the seeds being then thoroughly mixed. Employing 500 seeds from each sample so prepared, the percentage number of contaminated seeds was determined by the Ulster method. Two varieties, Liral Prince and Liral Crown, entered into the experiments made with each parasite. The seed was sown in drills 2 yd. in length and 1 ft. apart, 2·3 g. of seed being sown in each drill. Each seed sample was used for sowing a group of four drills, such groups being separated by a path 2 yd. in width in order to reduce the danger of transmission of spores from diseased plants to others resulting from the sowing of separate seed samples. Two plots were sown with seed from each sample, employing the same technique as was used in 1940. The seed was sown in both plots and drills on 22 May.

Table 3. *Relationship between contamination of seed with* Polyspora Lini *and the number of diseased plants in the crop*, 1942

Variety	Laboratory test % seeds contaminated with *P. Lini*	Field test Average % plants infected with *P. Lini*	
		Seedling phase	Stem-break
Liral Prince	0·2	0·5	2·3
,, ,,	0·6	0·2	1·5
,, ,,	0·8	1·5	3·9
,, ,,	1·6	0	3·1
,, ,,	1·8	2·9	8·7
,, ,,	3·6	1·1	7·9
,, ,,	9·8	1·3	14·6
,, ,,	15·6	4·8	40·7
,, ,,	31·4	10·1	39·1
Liral Crown	0·6	0·5	1·6
,, ,,	0·6	0·6	12·1
,, ,,	1·0	0	2·6
,, ,,	2·4	0·4	7·6
,, ,,	8·4	2·1	16·0
,, ,,	19·0	4·9	33·7

Table 4. *Relationship between contamination of seed with* Colletotrichum linicola *and the number of diseased plants in the crop*, 1942

Variety	Laboratory test % seeds contaminated with *C. linicola*	Average % plants infected with *C. linicola* Seedling phase
Liral Prince	1·6	1·2
,, ,,	2·0	6·2
,, ,,	2·2	2·9
,, ,,	2·4	0·9
,, ,,	2·4	2·6
,, ,,	2·6	2·0
,, ,,	3·8	7·1
,, ,,	6·8	3·4
,, ,,	9·4	3·2
Liral Crown	0·6	6·2
,, ,,	1·0	4·3
,, ,,	2·8	11·1
,, ,,	4·8	13·5
,, ,,	11·0	16·8
,, ,,	17·2	18·6

Since the crops in plots were damaged by drought in June, observations were not made on them. On 16 June the plants in two of the drills sown with each seed sample were pulled and the percentage number in each drill showing infection with *P. Lini* and *C. linicola* in the seedling stage was determined, all infected plants being subjected to microscopic examination. On 27 July the plants growing in the two remaining drills sown with each seed sample contaminated with *P. Lini* were pulled, and the percentage number of plants showing stem-break in each drill calculated. The average percentage number of plants showing infection with *P. Lini* in the seedling and stem-break phases for each two rows sown with the same seed sample is given in Table 3, together with the percentage number of contaminated seeds in each sample. The corresponding values for *C. linicola* relating to the contamination of the seed and the incidence of the seedling phase in the crops are presented in Table 4.

It can be seen from Table 3 that the percentage number of plants infected with *P. Lini* either in the seedling or stem-break phase is, in general, related to the percentage number of contaminated seeds among those sown. It should be noted that the percentage number of plants exhibiting stem-break was substantially higher in every case than the corresponding value obtained for the seedling phase of the disease, although the number of plants in the drills at the times when records were made showed no real difference. This result can be attributed to the spread of the disease occurring after the observations on the seedling phase were made.

In the case of seed contaminated with *C. linicola* agreement is also shown between the values for the contamination of the seed and the number of infected plants. The agreement is not so close as was found when *P. Lini* was studied, possibly because of *C. linicola* spreading more rapidly from plant to plant.

The writer wishes to express his thanks to Prof. A. E. Muskett for his interest and criticism and to the Agricultural Research Institute, Hillsborough, for the provision of field facilities. Acknowledgement is made to the Flax Development Committee for its generosity in continuing to provide a grant-in-aid to the Plant Disease Division in support of the investigation of diseases of flax.

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Bionomics of the carrot fly (*Psila rosae* Fab.)

II. Soil populations of carrot fly during autumn, winter and spring

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(With 7 Text-figures)

The behaviour of the carrot-fly population in the soil during autumn and winter has been investigated in East Anglia over the three seasons 1943–5. The results show that the pest overwinters in both the larval and pupal conditions, and that the proportion of the individuals in each stage varies from crop to crop and from season to season. Samples taken from a May (1943) sown plot showed that pupation, which was 45·2 % complete in October, rose to 74·2 % in March and was not complete until late April. From a June (1944) sown plot of carrots samples showed a rise in percentage pupation from 8·6 % in October to 93·5 % in March, the process probably being complete by early April. The changes in proportion of the soil larvae, carrot larvae and puparia in both the May and June plots were followed and are shown graphically. The changes in the size groups of the soil larvae and in the carrot larvae were also traced. The immature larvae persist longer in the carrots than in the soil. The size composition of the larvae from the soil of the June (1944) plot was recorded over the period September to April and the results are given graphically. Samples taken in October 1945 contained highly organized puparia from which flies emerged in a few days. These flies (third generation) gave rise to progeny which overwintered in the larval condition. The population in December 1945, composed chiefly of second generation puparia and third generation larvae, was very similar to that occuring in the previous two years. Mortality in the overwintering carrot-fly larvae and puparia varied considerably but was frequently heavy. The principal agencies concerned were an hymenopterous insect, *Dacnusa gracilis* Nees., and fungus and bacterial diseases. Other insect parasites occasionally important were *Loxotropa tritoma* Thoms. and *Aleochara sparsa* Heer. Nematode parasites were also recorded.

Introduction

The behaviour of the carrot-fly larvae during autumn and winter in relation to the duration of their various stages and times of pupation has for some time been a subject of controversy. From Washington, Mass. (U.S.A.), Hanson & Webster (1941) have reported that the flies overwinter both as larvae in the carrots and as puparia in the soil, and stress that mortality during the winter months is conspicuously high. Savzdarg (1927) states that in the Leningrad district (U.S.S.R.) the majority of overwintering puparia are produced from the first generation of flies, i.e. the fly is univoltine, and that a partial second generation also occurs. Larvae from this generation may pupate in autumn and early winter, but some do not pupate until the following May. Smith & Gardner (1922) from investigations near Manchester state: '...there are two generations of *P. rosae* in a season, but neither of these is complete, as many pupae from the first generation of maggots do not hatch that year, but pass the winter in the pupal condition. All maggots of the second generation pupate about the end of September and hatch in the following spring. In the exceptionally hot weather of the autumn of 1921, some second generation larvae were still feeding in carrots at the end of October.'

Observations made over three seasons have shown that in East Anglia the winter was passed in both the puparial and larval stages. The proportion in the puparial stage at the beginning of winter varied greatly from crop to crop. It was liable to be different for crops of different ages growing in the same locality, and of the same age growing in different localities; annual variations also occurred. The main factors causing these differences appeared to be the time of onset and the duration of the second generation attack. Emergence and sweeping records have shown that in the same locality these factors do show both a considerable annual variation and a variation correlated with the age of the crop and its proximity to a source of flies.

To throw more light on this matter the development of overwintering populations of larvae and puparia was followed in some detail on one plot of carrots in 1943, and another in 1944. Both were on black fen soil at Hall Farm, Mepal (Isle of Ely), whilst several other sites both at Mepal and elsewhere were sampled for checking and comparison. The date of sowing of each plot had a considerable effect on the intensity and progress of the attack in summer and autumn; the 1943 plot was sown on 10 May and suffered a heavy first generation attack beginning in the cotyledon stage, and a corresponding heavier attack commencing in August from flies of the second generation. The 1944 plot was June sown and experienced no first generation attack, but the second generation attack began in late August by migrant flies from a nearby field of maincrop carrots. The attack on this plot was heavy, flies being present and eggs being laid well into October. These plots will be referred to as May (1943) and June (1944) plots respectively.

In 1945 samples were taken from carrot plots and headlands of carrot fields in a number of localities in East Anglia. The season had been sufficiently early to allow many larvae from the second generation of flies to pupate by mid-September, and from these a third generation of flies began to emerge in late September and continued well into November. The headland of one field at Mepal, sown in June, experienced some attack in August from migrant second generation flies and itself gave rise to considerable numbers of third generation flies in October. The development of the progeny of these flies was followed by periodically sampling the headland during autumn and early winter. This sampling site will be referred to as the June (1945) headland.

Method of sampling

The method chosen was to remove from the plot or headland a number of 1 ft. lengths of carrot row and associated soil. Each unit sample was 1 ft. long, 6 in. wide and 6 in. deep, and was taken at random on the site. Investigations on the distribution of the puparia in the soil around carrots showed that samples of this size would contain 93% of the puparial population (Petherbridge & Wright, 1943).

From the May (1943) plot ten samples were taken at each date of sampling, sieved and graded separately. From the June (1944) plot, and all sites sampled in 1945, five samples only were taken from each site and these were bulked. This change in number and treatment of the samples resulted from a failure to derive from the May plot data additional information resulting from treating the ten replicated samples singly. The May and June plots were sampled at approximately monthly intervals from October to June 1943–4, and from September to April 1944–5. The June (1945) headland was sampled three times during the period October and November 1945. The soils were washed through two sieves, an upper one of about eight meshes to the inch to break up the soil and retain the carrots and weeds, and a lower one of sixty meshes to the inch which retains the smallest carrot-fly larvae and even the eggs. From the residue in the lower sieve the organic material was floated off in saturated brine and the carrot-fly larvae and puparia removed by hand picking. To avoid missing small larvae the samples were then boiled and re-examined. The boiled larvae are white and opaque and are conspicuous against a black background (for further details of this method of flotation see Cockbill *et al.* 1945).

CONSTITUTION OF THE SOIL POPULATION

The soil population of carrot fly comprises the egg, larval and puparial stages, but only the two latter will be dealt with here, since the egg stage is not present during winter or spring. Larvae in all stages of growth may be found in the soil, there being present at the same time young larvae which have only recently hatched and large fully grown larvae about to pupate, as well as larvae in intermediate stages of growth migrating from carrot to carrot. The larvae have been classified into the following three groups based on length: 0–3·5, 3·6–5·9 and 6·0–10·0 mm., and during 1944–5 the larvae from the June plot were measured individually to the nearest $\frac{1}{8}$ mm.

The numbers of puparia in the soil vary greatly from autumn to spring. When pupating the larva darkens, shortens and hardens its cuticle to form the characteristic cyclorraphous puparium, which varies in colour from pale yellow to chestnut brown. The newly formed puparia are soft and cream-coloured, and the frequency of these (called 'white pupae') gives an indication of the rate of pupation of the larvae. Although the puparium rapidly darkens and hardens, the internal larval organization is not rapidly destroyed and is visible without dissection for some time afterwards. Puparia in this condition together with 'white pupae' have been designated 'unorganised'. The older puparia have been classified into three arbitrarily selected stages, 'organized+', 'organized++', and 'organized+++'. The first stage comprises puparia in which the outer pupal skin is distinct with the legs and wing sacs prominent and the pupa surrounded by gas, giving the puparium a silvery appearance and enabling it usually to float on water. The second stage (organized++) is reached when the eyes begin to colour and continues until the third stage is reached, when the adult is clearly differentiated inside the pupal skin and a darkening of the adult cuticle has begun. Puparia formed in autumn and winter develop as far as the 'organized+' stage and remain in this condition, perhaps for several months until the spring, when they pass rapidly through the two remaining stages. In October 1945 puparia were found in the two highest stages of organization, but these either emerged in early winter or failed to survive.

There is a characteristic difference between the puparia formed in autumn and early winter and those formed in spring. The former are smooth and pale yellow with inconspicuous rings encircling the puparium and the segmentation is not clearly marked, whilst the spring-formed ones are generally larger and much darker in colour, and have characteristic heavily marked rings around the puparium both at and between the intersegmental points. Puparia formed during winter tend to be intermediate in size and form between these two types. As pupation does not proceed rapidly during winter the proportion of such intermediate types to the total puparial population is usually low.

A large number of puparia deviate from the normal (healthy) types outlined above and may often be recognized by external appearance. These are forms parasitized by other insects and to a less extent by nematodes, fungi and bacteria. An account of the identity and incidence of some of these will be given later.

THE CHANGES IN THE SOIL POPULATION, MAY PLOT, MEPAL, 1943–4

Ten samples, chosen at random, were taken each month from this May-sown plot of carrots at Mepal.

TABLE 1. *May plot*, 1943–4. *Changes in percentage composition of the soil population*

Date of sampling	Carrot larvae %	Soil larvae %	Puparia %	Total individuals in 10 unit samples
19 Oct.	25·2	29·6	45·2	1313
16 Nov.	29·7	11·5	58·8	1269
16 Dec.	22·4	11·7	65·9	993
11 Jan.	35·0	9·0	56·0	1076
8 Feb.	23·4	15·2	61·4	884
23 Feb.	18·4	18·9	62·7	884
14 Mar.	7·7	18·1	74·2	1086
4 Apr.	2·1	3·6	94·3	782
24 Apr.	0	0·9	99·1	931
25 May	0	0	100·0	918
19 June	0	0	100·0	955

In Table 1 is given the total numbers of individuals in the ten unit samples for each date, and also the percentages in the puparial and larval stages. The latter are divided according to their location in the sample into 'soil larvae' (found in the soil) and 'carrot larvae' (found in the carrots). Statistical analysis of the data from the ten unit samples suggested that pupation had proceeded rapidly during the period 19 Oct. to 16 Nov. and also in the spring (1944) from 23 Feb. to 25 May. Between 16 Nov. and 23 Feb. no significant ($P=0·05$) change in the percentage of puparia in the samples was indicated. However, rearing experiments in the field with material from this plot showed that during the period 2 Dec. to 15 Feb. some 20% of the carrot larvae had left the roots and formed puparia. Therefore to maintain an apparently unchanged puparial percentage a loss of puparia must have occurred of approximately double the number added by pupation during the period. Losses of puparia by fungal and bacterial decay and from predators is known to be heavy in certain seasons. Sampling was continued until some 6 weeks after the start of emergence of the spring generation of flies, and the last two records

showed the presence of large numbers of empty puparia.

The changes in proportion of the population partitioned into soil larvae, carrot larvae and puparia are shown graphically in Fig. 1, based on the data given in Table 1. Curve *A* gives the changes in the total larvae as a percentage of the population and shows a downwards trend to 24 Apr., when only 1% of the population was in the larval stage; not until 25 May had all the larvae disappeared. The larvae in the soil are shown by curve *B*. Their proportion decreases rapidly from 19 Oct. to 16 Nov., and this is accompanied by a significant increase in the percentage of puparia and also by a rise in the percentage of larvae in the carrots, represented by the vertical distance between curves *A* and *B*. The percentage of puparia in the population is represented by the vertical distance between curve *A* and the

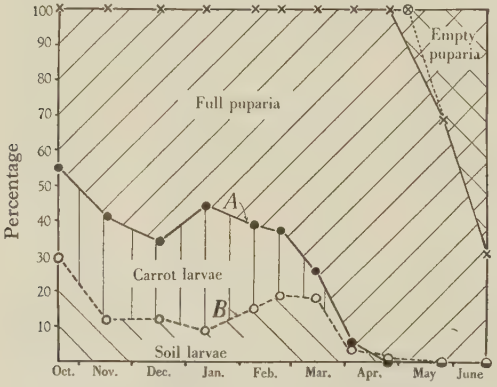


Fig. 1. Composition of the soil population, May (1943) plot, Mepal.

upper limit (100%) of the graph. After falling to a low value in January the percentage of soil larvae rose until March, after which date pupation was rapid and the larvae in the soil and in the carrots soon disappeared. The highest figure for percentage carrot larvae was in January, corresponding with the minimum for percentage soil larvae, and the increasingly rapid fall in number of the carrot larvae during February, March and April is shown by a convergence of the two curves. On 24 Apr. no larvae remained in the carrots and only 1% of the total population was in the larval stage. Inset at the top right-hand corner of the graph is the percentage of empty puparia, denoting adult emergence which began on 5 May and continued until early August at a gradually decreasing rate.

Changes in the size groups of the larvae in the soil and in the carrots were also followed. Larvae were graded into three groups according to length (0–3·5, 3·6–5·9 and 6·0–10·0 mm.) and the two lower size groups together denoted 'immature larvae'. In Fig. 2, curve *A* shows the immature larvae in the soil as a percentage of the total larvae in the soil, and curve *B* shows the immature larvae in the carrots as a percentage of the total larvae in the carrots. Fig. 3 shows similar ratios for the smallest size group (0–3·5 mm.) of larvae in the same two situations.

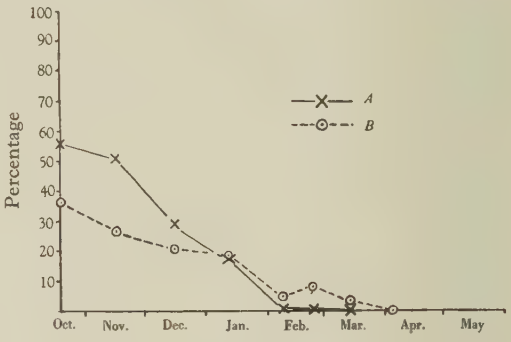


Fig. 2. May (1943) plot, Mepal. Immature larvae in the soil and in the carrots.

Curve *A* = % of larvae in the soil immature (0–5·9 mm.).
Curve *B* = % of larvae in the carrots immature (0–5·9 mm.).

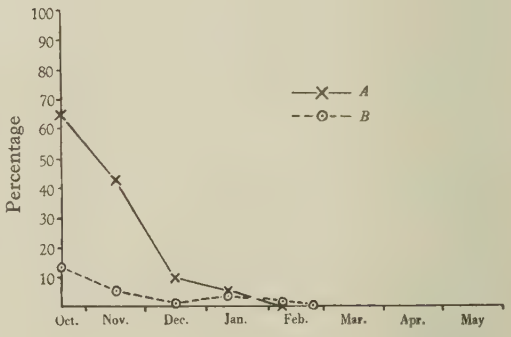


Fig. 3. May (1943) plot, Mepal. Very small larvae in the soil and in the carrots.

Curve *A* = % of larvae in the soil very small (0–3·5 mm.).
Curve *B* = % of larvae in the carrots very small (0–3·5 mm.).

For both size groups the larvae persist longer in the carrots than in the soil as shown by the crossing over of the two curves. Small larvae were last recorded in the carrots on 8 Feb. but in the soil only until 11 Jan. Whether the latter had previously been feeding in the carrots or only on the lateral roots and fragments in the soil is not known. The rapid decrease in immature soil larvae during autumn

agrees with the observed rapid deterioration of carrots left in the ground during autumn and winter in the presence of high larval populations of carrot fly (see Wright & Ashby, 1946).

CHANGES IN THE SOIL POPULATION, JUNE PLOT, MEPAL, 1944–5

Records were obtained from this June sown plot at approximately monthly intervals from September to May 1944–5. At each date five unit samples only were taken and examined together, thus providing only one set of data for each time of sampling. The plot received no first generation attack and the onset of the second generation was much later than on the May plot in the preceding year. The population on the June plot was therefore comparatively young when sampling commenced, and contained only 8·4 % of puparia as compared with 45·2 % in the

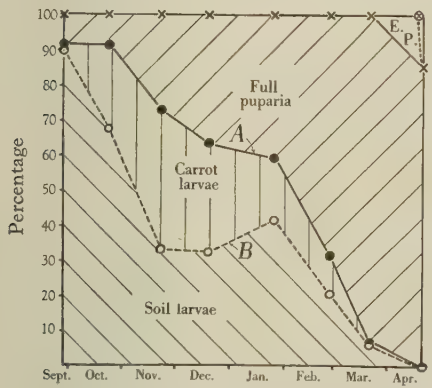


Fig. 4. Composition of the soil population, June (1944) plot, Mepal.

preceding year. Fig. 4 shows the changes in the percentage composition of the population. The numbers involved were approximately half those obtained for the May plot, the difference being related to the size of the sample. Although the total numbers of individuals remained fairly constant throughout there was some evidence of their decline, due to puparial mortality during March and April. The diagram is directly comparable and shows a general similarity to that for the May plot (Fig. 1). The soil larvae again show a low value in December and January, and this is followed by an increase in numbers as larvae leave the carrots prior to pupation. The decreasing percentage of larvae in the carrots is reflected in the convergence of curves *A* and *B*.

There are, however, several noteworthy points of difference between the two years' results. Sampling of the June plot was begun a month earlier than on the May plot, at a time when there were practically no larvae in the carrots. Comparison of the second sample of the June plot with the first of the May plot (both samplings taken about the third week of October) shows that migration of the larvae from the soil into the carrots had, by that date, proceeded much further in 1943 than in 1944. This appears to

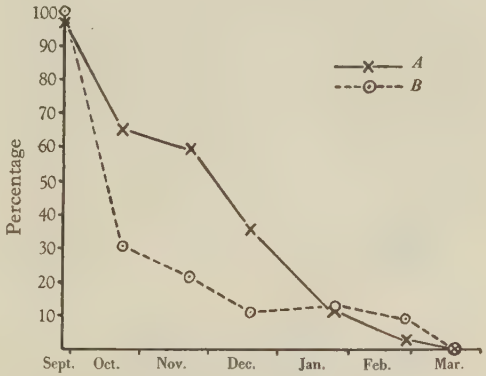


Fig. 5. June (1944) plot, Mepal. Immature larvae in the soil and in the carrots.

Curve *A* = % of larvae in the soil immature (o–5·9 mm.).
Curve *B* = % of larvae in the carrots immature (o–5·9 mm.).

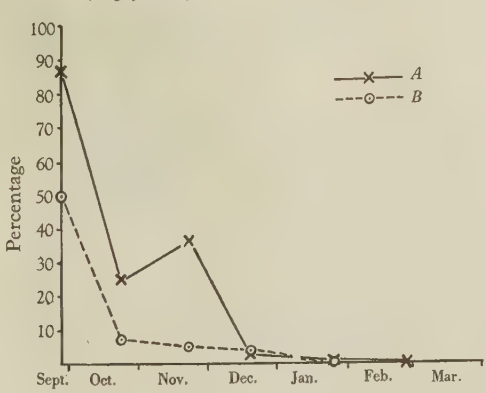


Fig. 6. June (1944) plot, Mepal. Very small larvae in the soil and in the carrots.

Curve *A* = % of larvae in the soil very small (o–3·5 mm.).
Curve *B* = % of larvae in the carrots very small (o–3.5 mm.).

be due entirely to the fact that the May plot received a heavy attack at an earlier date than the June plot. Comparison of the results obtained in March, April and May show considerable differences to occur in the two years. In 1944 (Fig. 1) a much higher percentage of the population was in the larval stage during March than in 1945 (Fig. 4). Also, in the

former, larvae were present during April, whilst the June plot gave 100% puparia in the sample taken 20 Apr. These differences do not appear to be connected either with the relative sowing dates or with the time of the second generation attack. Both these features, in fact, might have been expected to have resulted in an earlier pupation on the May sown plot. That this was not so appears to be due to weather differences between the two years. Jan. 1944 was a very mild month in a generally mild winter, but was followed by a cold period during late February and in March. Conditions in 1945 were the reverse of this, with January very cold and

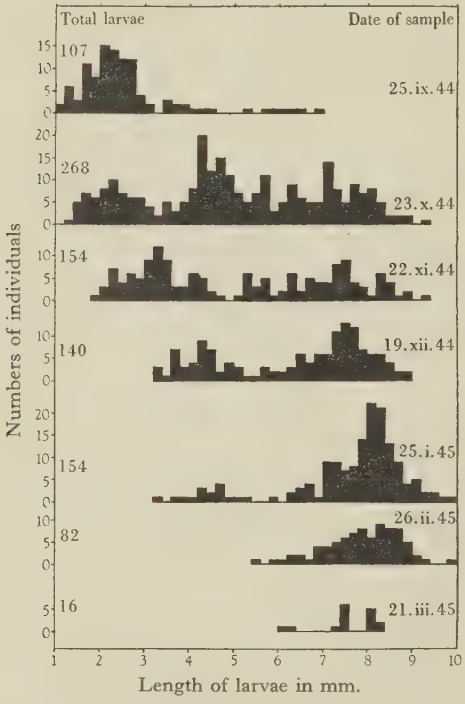


Fig. 7. Size distribution of soil larvae in June (1944) plot, Mepal.

February and March very mild. It appears probable that the relaxation of the cold conditions at the end of January allowed development to proceed at a rapid rate during February and March, whilst maturation of the population over this period in 1944 was checked by low soil temperatures. The earliness of insect emergence in 1945 was abundantly noted in the field; carrot flies began to emerge in the Mepal area on 17 Apr., some 18 days earlier than in the preceding year.

The size groups of the larvae in the soil and in the carrots were followed as in 1943–4, and these are shown, for the immature and very small groups, in Figs. 5 and 6 respectively. The curves for soil larvae and carrot larvae are again seen to cross over, larvae tending to be present for a longer period in the carrots than in the soil.

The soil larvae from this June plot were also measured to the nearest $\frac{1}{5}$ mm. The results, for each date of sampling, are graphed as histograms in Fig. 7. In each case the areas are proportional to the total numbers present. The initial increase in numbers (resulting from the hatching of eggs) and the decline through the last three samples is clearly shown. The growth of the population is also shown by the steady shift of the peak towards the right of the graph. Mutilation of some of the larvae from the carrots prevented the construction of a similar growth diagram for the carrot larvae. It is probable, however, that the changes in size of these larvae would fall into a very similar pattern to those from the soil, there being a steady decrease in the size range of the larvae found in the carrots and a gradual shift of their peak numbers towards the larger size groups.

The presence of half-grown larvae in considerable numbers in the soil as late as the end of January affords more evidence for the migration of larvae from root to root as hatching of the eggs will have ceased by early winter.

Soil populations, autumn, 1945

Samples were taken from a number of carrot plots and carrot fields in East Anglia during the period October to December 1945. These showed that, in all localities, the soil population in October was very different in composition from that in October of the two preceding years. In all cases the majority of the population were puparia and the larvae were mostly fully grown. Among the puparia, individuals in the two highest stages of organization (organised + + and + + +) occurred in all samples. These had not previously been found at this time of year, and when placed in a cool insectary gave rise to adults by the beginning of November or failed to survive the winter. These populations represented a part of the second generation, the remainder having emerged (as third-generation adults) during the autumn. The proportion which emerged during autumn varied from crop to crop and with the locality. From an assessment of empty puparia it was found to be never more than 50% and usually between 15 and 20% of the total population. The progeny of the third generation of flies (on the wing in October and early November) greatly increased soil populations by mid-November, and many larvae at this time were immature. The population changes occurring on the headland of a June sown field at Mepal are shown in Table 2.

The sample taken on 27 Nov. showed that the larvae had grown considerably in the preceding

12 days. Although this site could not be resampled, data obtained from other plots showed that by mid-December the size distribution of the larvae in the soil and in the carrots was similar to that occurring in mid-December 1943 and 1944. Thus, aided by a mild autumn, the progeny of the third-generation flies reached stages of development in December comparable with those reached by larvae of the second generation in the years 1943 and 1944. These populations, containing in all cases a high percentage of puparia, were similar in composition at the onset of winter to that on the May (1943) plot at that time. They were, however, as overwintering populations, atypical in origin, since emergence records over the previous five years (1940–4) showed no evidence of a third generation.

Table 2. *June* (1945) *Headland. Changes in composition of the soil population*

Date of sampling	Total individuals	Total larvae	Size distribution of larvae from soil and carrots (%) 0–3·5 mm.	3·6–5·9 mm.	6·0 mm. and over	Puparia %
16 Oct.	58	13	0	6·7	93·3	77·6
15 Nov.	191	106	18·7	27·0	54·2	44·7
27 Nov.	193	117	5·1	28·2	66·7	39·4

Mortality in the overwintering carrot-fly population

The overwintering populations of carrot fly are subject to attack by a number of parasitic organisms. Among the most important of these are two species of hymenopterous insects, both of which are larval parasites and pass the winter in the larvae or puparia of the host. Both are widespread in East Anglia, and of these *Dacnusa gracilis* Nees. (Braconidae, Dacnusinae) is numerically the more important. Parasitism by this insect has been followed in the Mepal (Isle of Ely) area and at the Field Station, Cambridge, over the period 1943–5. In both places parasitism of the overwintering puparia normally varied from 20 to 40%. Although values outside this range were occasionally obtained the results in general suggest that where carrots are grown frequently on adjoining or neighbouring land this level of parasitism of the overwintering puparia can be expected. That it is possible to increase this parasitism by intensive breeding of the host insect was indicated by data obtained at Swaffham. Here a succession of carrot sowings was made on the same plot of land in 1944 and in 1945. The overwintering puparial parasitism in 1944 was 24·4%, and this rose steadily throughout the following year and reached 62·8% in October 1945. This level of parasitism was, however, not sufficient to prevent severe damage to the carrots grown there.

The second hymenopterous parasite *Loxotropa tritoma* Thoms. (Proctotrypoidea, Diapriidae) is less common numerically than *Dacnusa gracilis*, and was sometimes absent from soil populations examined in winter. The highest parasitism of the puparia recorded at this time was 11·4%.

Very sporadic in appearance and irregular in distribution on carrot fly is *Aleochara sparsa* Heer. (Staphylinidae, Aleocharinae). The larva of this beetle is endoparasitic in the puparium and has been recorded only from Mepal. Its maximum abundance was recorded in May 1944, when it was present in some 5% of the population at Mepal.

A more detailed account of the incidence and biology of these insect parasites will be given elsewhere.

Fungus and bacterial diseases play a considerable part in reducing the numbers of larvae and puparia in the soil. One disease causes browning and death of the larvae or puparia, and another takes the form of chocolate brown spots on the outside of the larva which eventually merge and cause extensive necrosis of the tissue. Insect-parasitized and unparasitized individuals are both attacked. The incidence of these diseases in normally low but occasionally reaches 10% of the population. As the diseased individuals decompose in the soil in a few weeks the loss is cumulative and results in a decrease in total population during winter. The losses incurred on the May (1943) and June (1944) plots on fen soil where the populations dropped in each case by some 30% over the period November to April can largely be ascribed to these agencies. In medium loam and sandy soils these losses, however, appear to be much less. Parasitism of the larvae and puparia by nematodes has also been recorded for most of the localities sampled. Two distinct types of worm differing greatly in size are involved. The larger types, measuring up to 30 mm. in length, are found singly or in pairs and occupy almost the whole body space of the host. The second form comprises numerous worms about $\frac{1}{8}$ mm. in length and found in all parts of the body cavity. Parasitism by these is generally less than 1% of the total population, but on the Swaffham plot in October 1945 it reached 28·5 and 2·9% of the larval and puparial populations respectively.

An analysis of the parasitism in the total population on the May (1943) plot for samples taken in April and May is given in Table 3.

Parasitism in the June (1944) plot and in samples from a number of localities in 1945 are shown in Table 4.

The samples were taken in October and November and the data show the relative parasitism by insect and by other agencies on the puparial and larval stages. The very high total parasitism recorded at Swaffham is not typical of the area but occurred on the small plot which had been intensively cropped with carrots.

Conclusion

A study of the soil populations of the carrot fly has shown that in East Anglia the winter is passed both in the puparial and larval stages. Many of the larvae are immature at the onset of winter but mature and pupate by the spring. Mortality during winter varies considerably but never appears to be sufficiently high to imperil the survival of the population.

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Table 3. *Analysis of parasitism in the total population. May* (1943) *plot*

Date of sampling	Total puparia	Percentage puparia attacked by: *Dacnusa gracilis*	*Loxotropa tritoma*	*Aleochara sparsa*	Fungi and bacteria	Nematodes	Total parasitism
24 Apr.	923	19·6	4·9	1·8	9·6	0·7	36·5
25 May	918	17·3	7·7	5·1	10·0	0·5	39·5

Table 4. *Parasitism of the larvae and puparia by insects and by other agencies. Samples taken October and November*

Locality	Puparia (*a*) Parasitism by insects %	Puparia (*b*) Parasitism by other agencies %	Larvae (*c*) Parasitism by insects %	Larvae (*d*) Parasitism by other agencies %	Average parasitism in the total population %
June plot, Mepal (1944)	22·9	0	5·7	4·7	15·7
Mepal (1945)	26·1	4·3	10·0	0	24·5
Cambridge (1945)	32·8	2·4	15·7	0	26·8
Swaffham (1945)	62·8	5·8	57·2	28·5	78·5
Kirton, Lincs. (1945)	29·3	0	15·4	0	26·4

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The comparative toxicity of various contact insecticides to the louse (*Pediculus humanus* L.) and the bed-bug (*Cimex lectularius* L.)

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A variety of substances has been tested by laboratory methods as insecticides against the bed-bug and the body-louse. The oil-soluble samples were tested by the Potter tower method which gave the most accurate results. Certain liquids were tested by a simple dipping method, and solids insoluble in mineral oils were tested as aqueous solutions by the dipping method or as dusts.

Various organic thiocyanates were found to show similar relative toxicity to the louse and bug, but the pyrethrins were much more toxic to the latter. D.D.T. was highly toxic to both and Gammexane even more insecticidal, especially to the louse.

In the dipping tests, two types of toxic action were observed: an immediate physico-chemical action and a delayed lethal action due to asphyxiation. Refined mineral oils kill lice by the latter method. Among the aqueous preparations, the emulsion of cresols known as Lysol was noteworthy for its ovicidal powers.

Some experiments were made with extracts of delphinium seeds, the insecticidal powers of which were shown to reside in the alkaloids.

Introduction

This paper records some quantitative data on the toxicity of several groups of contact insecticides to lice and to bed-bugs. The value of such results from both the practical and theoretical standpoints is as follows:

(*a*) Laboratory experiments under controlled conditions give an accurate guide to direct insecticidal values.

(*b*) The results may provide initial data for investigations into the mode of action of insecticides, with special reference to (i) the toxicity of allied compounds in relation to their structural formulae and to their chemical and physical properties, and (ii) anomalies in the relative resistance of different types of insect to various toxicants (vide Busvine, 1942).

Technique

A. *Maintenance of insect stocks*

The lice were reared in gauze-bottomed pill boxes kept against the skin of the leg during the day as described by Buxton (1939). The bed-bugs were kept in muslin-topped glass tubes in an incubator at 23° C.; they were allowed to feed weekly on the ears of a lop-eared rabbit.

B. *Experimental methods*

Owing to the different physical properties of the variety of substances examined, it was necessary to employ three separate test methods.

(1) *Spray tests.* Oil-soluble substances were dissolved in refined white oil* and sprayed on to the insects in the apparatus described by Potter (1941). The insects were exposed to the spray in small glass dishes with the bottoms lined with filter-paper to absorb excess spray.

The concentrations of the toxicants were varied, while the volume of liquid was kept constant at 1 c.c. This was delivered by a hypodermic syringe and gave an average deposit of 0·36 mg./sq.cm. At this rate the oil carrier was completely harmless to the insects.

A small number of spray tests was made with aqueous solutions and emulsions. As some of the substances tested in this form were of low toxicity, a considerably larger volume was sprayed giving five times as heavy a deposit. Otherwise the technique was the same.

(2) *Dip tests.* The dipping tests were made only with lice and their eggs. The lice were dipped while clinging to small pieces of tape, and the eggs were dipped attached to the tapes on which they had been laid. At the end of the exposure they were removed, dried on blotting paper and aired for 5 min. in a stream of air passing at the rate of 20 m./min.

(3) *Dust tests.* Solids of low solubility in both oil and water were tested as dry powders, either 'neat' or diluted with kaolin. For the tests with lice, the samples were rubbed into pieces of tape, the excess flicked off and the tapes put into feeding tins with the lice. In the bed-bug tests, the insects were shaken up with the powders and then allowed to crawl on to paper slips; these papers were tapped briskly to remove excess dust.

C. *Estimation of results*

After the insects had been treated by any of the above methods, the lice were put into pill boxes and

* Oil P31 was used: Sp.gr. at 60° F., 0·862; I.B.P. 613° F.; flashpoint, closed, 365° F.; viscosity, Redwood I, 141 sec. at 68° F.

worn on the leg, while the bugs were put into clean tubes in an incubator at 20° C. The lice were examined daily and the final mortality determined 3 days after spraying; the bugs were examined after 7 days when the numbers of dead were counted. (The longer interval was necessary with the bugs because they did not die or recover so rapidly at 20° C. as the lice on the leg which were at, approximately, 30° C.)

The most precise results were yielded by the spray method which gave concentration-mortality relations which were plotted on log concentration-probit graphs. Median lethal concentrations were estimated from these curves either visually or by computation as suggested by Bliss (1935).

The results from the dip tests and from the dust tests were sufficiently reliable to indicate the degree of toxicity of the samples and place them in order of effectiveness. These data did not, however, permit accurate comparisons on the basis of the ratios of equitoxic doses (or concentrations). For that reason the spray method was employed wherever possible. Fortunately, most highly toxic insecticides are oil-soluble.

Presentation of results

The materials tested fall into two groups, and may be regarded as the subjects of two investigations undertaken with the same technique. The groups are

(A) Miscellaneous contact insecticides.

(B) Oils and alkaloids extracted from delphinium seeds.

Section (A). Miscellaneous contact insecticides

(1*a*) *Spray tests* (*oil-soluble substances*)

(i) *Samples used*

Pyrethrum: an extract containing 1·3% pyrethrins in white oil was used. This is supplied by Messrs Stafford Allen under the name Pyremist L.

iso*Butyl undecyleneamide*,

$$CH_2{:}CH(CH_2)_8CONH.CH_2CH(CH_3)_2{:}$$

is a yellowish waxy solid, melting at about 25° C. and readily soluble in mineral oils. Though not itself very toxic, it has been used to enhance the toxicity of pyrethrins to houseflies (Weed, 1938).

Gammexane: is the commercial name of an insecticide now being produced by Imperial Chemical Industries Ltd., of which the active principle is the γ isomer of hexachloro*cyclo*hexane. (The other isomers are very much less insecticidal.) A sample of pure γ-$C_6H_6Cl_6$ was used for this investigation. It is a white solid, melting at 112° C. and sparingly soluble in mineral oil; for further details see Slade (1945).

D.D.T., $(p\text{-}C_6H_4Cl)_2CH.CCl_3$: another synthetic insecticide, also a white crystalline solid (m.p. 107° C.). It is fairly soluble in many organic solvents but only to the extent of 2–3% in refined mineral oils and practically insoluble in water. A refined sample was supplied by the Geigy Company.

The Lethanes: Lethane is a generic commercial name for certain complex organic thiocyanates. They are all made in the U.S.A. and marketed in this country by Lennig and Co., from whom samples were obtained.

Lethane 384 is 50% *beta*-butoxy-*beta'*-thiocyano-diethyl-ether, $C_4H_9O(CH_2)_2O(CH_2)_2CNS$, and 50% odourless kerosene.

Lethane 60 is 50% *beta*-thiocyanethyl-laurate, $C_{11}H_{23}COO(CH_2)_2CNS$, and 50% odourless kerosene.

Lethane 384 *Special* is a 1 : 3 mixture of Lethane 384 and Lethane 60.

Lauryl thiocyanate: a commercial grade of dodecyl-thiocyanate, $C_{12}H_{25}SCN$, which is an oily liquid (D^{20} 0·89; vapour pressure at 38° C., 0·006 mm. Hg) miscible with mineral oils. The sample used was prepared by Imperial Chemical Industries Ltd., and contained 91% thiocyanate.

bis-*Ethyl xanthogen*,

$$C_2H_5OCSSCOC_2H{:}$$
$$\quad\quad\|\quad\quad\|$$
$$\quad\quad S\quad\quad S$$

is a yellow crystalline solid, melting at about 30° C. and readily soluble in mineral oils. It has a rather strong unpleasant smell. As substance '*K*' it has been used by the Russians to impregnate clothing and render them 'louse-proof' (Soboleva, 1942), and the Germans have also employed it in this way (Rose, 1943).

Thanite: a trade name for the thiocyanacetate of a secondary terpene alcohol (? fenchyl) which is said to be effective against many domestic insect pests (Anon. 1942). It is a pleasant-smelling brown liquid, miscible with mineral oils. It is manufactured in the U.S.A. by the Hercules Powder Company.

Benzyl benzoate, $C_6H_5CH_2O.COC_6H_5$: a colourless liquid with a rather pleasant almond-like smell. In the form of an emulsion it has been used with success for treating scabies (Mellanby *et al.* 1942). It has also been used against head lice (Blackstock, 1944).

(ii) *Results*

Results of tests with those materials are assembled in Tables 1 and 2. It may be of interest to compare some of these results expressed as μg. per insect and as mg./kg. insect weight. The figures in Table 3 were calculated from the outline area and weights of the two insects.

Some spray experiments were also made with louse eggs of mixed ages. (Recently laid eggs were found to be more susceptible than older ones.) The

median lethal concentrations (M.L.C.) were determined graphically for a few substances, thus:

	M.L.C.
D.D.T.	Over 3% (saturated solution) (8% kill only)
Lethane 384	6%
Lauryl thiocyanate	18%
*bis*Ethyl xanthogen	Over 50% (30% kill only)
Benzyl benzoate	Over 50% (40% kill only)
Thanite	Over 50% (15% kill only)

(iii) *Comments*

There are a number of differences in the relative toxicity of the various insecticides towards lice and towards bed-bugs. In order to make a valid comparison it is necessary to take some results as a basic standard. The figures obtained with the various thiocyanates will serve this purpose with the present results.

Thiocyanates: the median lethal concentrations of these drugs are in the same order with both insects. The figures for the bug are 3–5 times those for lice. (The variation in the ratio may, perhaps, be due to experimental errors.)

TABLE 1. *Results of spray tests with body lice*

n = number of experiments; $n-2$ = degrees of freedom. Overall average number of insects per experiment = 40. χ^2, which measures the goodness of fit of the observations to the regression equation, was calculated as suggested by Bliss (1935).

Insecticide	Median lethal concentration (%)	Regression equation	$n-2$	χ^2	P (app.)
Gammexane	0·016	$y=3·6271x+0·6538$	3	0·7589	0·85
Pyrethrins (in the presence of 2% *iso*-butyl undecyleneamide)	0·038	$y=2·1894x+4·5768$	2	0·6230	0·75
D.D.T.	0·30	$y=4·5906x+7·1162$	2	1·4859	0·6
Pyrethrins	0·47	$y=3·3532x-0·2422$	4	1·8139	0·85
Lethane 384 (active principle)	1·5	Insufficient points	1	—	—
Lethane 384 Special (active principle)	2·4	$y=4·5163x+3·2548$	2	0·4588	0·75
Thanite	3·2	$y=2·0848x+3·9542$	3	5·0961	0·15
Lauryl thiocyanate	6·0	$y=4·0265x+1·8591$	4	1·3240	0·85
*bis*Ethyl xanthogen	6·2	$y=3·8243x+1·9529$	3	0·1240	0·98
Lethane 60 (active principle)	8·1	$y=3·2475x+2·0318$	4	2·9233	0·6
Benzyl benzoate	21	$y=4·4016x+0·8683$	3	1·0044	0·8

TABLE 2. *Results of spray tests with bed-bugs*

n = number of experiments; $n-2$ = degrees of freedom. Overall average number of bugs per experiment = 30. χ^2, which measures the goodness of fit of the observations to the regression equation, was calculated as suggested by Bliss (1935).

Insecticide	Median lethal concentration (%)	Regression equation	$n-2$	χ^2	P (app.)
Pyrethrins (in the presence of 2% *iso*-butyl undecyleneamide)	0·026	$y=2·1976x+4·3651$	2	1·1893	0·6
Pyrethrins	0·045	$y=3·8951x+2·8111$	5	3·3917	0·6
Gammexane	0·051	$y=3·900x+2·2075$	3	7·4988	0·07
D.D.T.	0·56	$y=2·9353x+5·6122$	2	8·7885	0·015
Lethane 384 (active principle)	4·0	$y=2·8590x+3·3114$	4	1·1281	0·85
Lethane 384 Special (active principle)	12·5	$y=3·9997x+0·6046$	5	3·8800	0·6
Lauryl thiocyanate	19·5	$y=4·9146x+1·3435$	3	2·5071	0·4
Lethane 60 (active principle)	32	$y=3·9308x+0·9090$	2	0·4480	0·75
Thanite	75	Insufficient points	0	—	—
*bis*Ethyl xanthogen	75	Insufficient points	0	—	—
Benzyl benzoate	75	Insufficient points	0	—	—

TABLE 3. *Median lethal doses expressed as micrograms per insect and milligrams per kilogram (live weight)*

	Median lethal dose			
	μg. per insect		mg./kg.	
Insecticide	Lice	Bugs	Lice	Bugs
Gammexane	0·003	0·023	1·5	6
D.D.T.	0·054	0·25	27	63
Pyrethrins	0·085	0·020	42	5
Pyrethrins (+I.B.U.)	0·007	0·012	3·5	3
Lethane 384	0·27	1·80	135	450

	M.L.C.		
	Lice %	Bugs %	Ratio
Lethane 384	1·5	4·0	×2·7
Lethane 384 Special	2·4	12·5	×5·1
Lauryl thiocyanate	6·0	19·5	×3·2
Lethane 60	8·1	32	×3·9

Pyrethrins: in relation to the above standard, pyrethrins are very highly toxic to the bed-bug.

The adjuvant *iso*butyl-undecyleneamide greatly enhances the toxicity of pyrethrins to lice but has little effect on the already low dose for bugs.

New synthetics: Gammexane is the most toxic synthetic insecticide in this series; it is especially active against lice, being even more toxic than synergized pyrethrins. D.D.T. is also highly toxic compared with other synthetic insecticides.

Other insecticides: Thanite and *bis*ethyl-xanthogen, which are moderately toxic to lice, are comparatively ineffective against bed-bugs under these conditions. Benzyl benzoate is of low insecticidal power against both insects.

(1*b*) *Spray tests* (*water solutions and emulsions*)

(i) *Samples used*

Formaldehyde was the only substance tested as a solution. The other samples were emulsions prepared by diluting the following concentrates:

Lysol:	Liquor cresoli saponatus, B.P.
Lauryl thiocyanate:	25% lauryl thiocyanate, 5% triethanolamine stearate and 75% water.
Rotenone:	1% rotenone (in 7% derris), 30% castor oil emulsified in water ('Liquid Lice Preparation' obtained from Messrs Cooper, MacDougal and Robertson).

(ii) *Results*

Lice and their eggs were sprayed with four to seven concentrations of the various substances at the rate of 1·8 mg. of liquid per sq.cm. M.L.C. were estimated graphically and are shown in Table 4.

Table 4. *Results of spray tests, against lice and nits, of aqueous preparations*

Substance	Approximate median lethal concentrations: Lice (%)	Eggs (%)
Rotenone	0·15	—
Lauryl thiocyanate	0·5	1
Formaldehyde	9	25
Lysol	12	5

(iii) *Comments*

Even allowing for the heavier deposit, lauryl thiocyanate seems to be more toxic as an emulsion than as a solution. The higher toxicity of lysol to eggs than to adult lice is noteworthy (see Scobbie, 1945).

(2*a*) *Dip tests* (*non-aqueous liquids*)

(i) *Samples tested*

Kerosene (paraffin) and xylol have often been used for head and crab lice, the former mixed with bland vegetable oil and the latter with vaseline to reduce skin irritation.

Perchlorethylene, trichlorethylene and white spirit are fluids used in 'dry-cleaning' and were tested to determine whether this process could be relied upon to disinfest clothing. It will be seen that they rapidly kill the active stages of lice, but their effect on the eggs in a short exposure is doubtful.

Kerosene and white spirit are petroleum-oil fractions, and a range of other fractions was included for comparative purposes. Crude kerosene (burning oil) contains various aromatic substances which give the persistent unpleasant smell. The degree of refinement of these oils (i.e. extraction of aromatics) is indicated by the unsulphonatable residue (see Table 5).

(ii) *Results*

Results of the dip tests with the petroleum oils are given in Table 6.

(iii) *Comments*

Lice examined under a medium-powered binocular microscope during their period of immersion in various liquids show different types of toxic action. Some of the liquids rapidly cause obvious signs of injury, apparently by a physical action: all volatile fat-solvent liquids belong to this class. Refined paraffin oils of high boiling-point, on the other hand, act in a purely mechanical way by occluding the respiratory system. Immersion in these liquids produces the same effects as deprivation of oxygen, which can be observed by immersing the insects in a stream of coal gas. Major movements cease, but peristalsis continues for an hour or two* and no gross pathological changes are observed for many hours (e.g. the gut does not rupture for at least 12 hr.).

Death from asphyxiation is slow, for lice may sometimes revive after 24 hr. immersion in water. Therefore the dipping time is of little importance except in so far as it affects the degree of occlusion of the tracheae. The amount of drying and airing given subsequently is equally important; very careful cleaning is necessary to revive adult lice after immersion in white oil or medicinal paraffin. This is also true for the eggs. As Bacot & Lloyd suggested in 1919, the ovicidal action of kerosene is largely due to residual traces. Since this depends so much on drying and airing, the 'lethal' dipping times for eggs are omitted from Table 3 for the mechanically acting liquids.

* Peristalsis persists for much longer during immersion in refined kerosene than during immersion in water. This is probably because the solubility of oxygen in kerosene is 4½ times that in water, so that the insect may be able to continue slight movements by cuticular respiration (see Richards (1941) on mosquito larvae and paraffin oils).

Even a preliminary treatment may affect the results of dipping adult lice. If they are immersed for a short time in alcohol and rapidly dried, the alcohol forms a plug in the main spiracle trunks* and prevents the rapid entry of other liquids. Some of the insects will then survive an immersion in kerosene or white spirit which would otherwise be fatal.

Ashner & Mager (1945) point out that first-stage nymphs survive a much longer exposure in certain liquids than adult lice. This is contrary to the usual experience in insecticidal tests on lice and bugs. The present results confirm their observation, however, and allow an explanation to be offered for the anomaly. It will be seen in Table 3 that the only substances to which nymphal lice are markedly more 'resistant' than adults are the mechanically acting liquids. There seem to be two reasons for this. The higher, more viscous members of the paraffin series (white oil and medicinal paraffin) do not so easily invade the finer tracheae of the nymphs and, if the latter are wiped dry after immersion, they are much less liable to asphyxiation than the adults, whose wider tracheae are much more easily filled. Kerosene, certainly, penetrates both stages rapidly and completely: but a few hours later it can be seen to have partially dried out from the smaller bodies of the nymphs, and a day later their drying and revival is often complete. The tracheal system of the adult remains almost entirely clogged.

The liquids which are assumed to possess a physical toxic action apparently kill the insects during the period of immersion. The peristaltic movements of the gut cease in a short time—usually less than 5 min. Soon afterwards the skeletal muscles turn milky white and gradually become more or less opaque, presumably owing to coagulation of the protoplasm. About 2 hr. after the dipping, the wall of the gut breaks and liberates the contents into the haemocoele. Some of these substances do not enter the tracheal system but must penetrate the general cuticular surface.

It will be seen in Table 3 that some of the unrefined kerosene samples are not purely mechanical

TABLE 5. *Specifications of petroleum-oil fractions used in the dipping tests with lice*

Name	Boiling range ° C.		Unsulphonated residue	Sp.gr.	Viscosity (20°)	
	Initial	Final	%	(16° C.)	Redwood I (sec.)	Centistokes
n-Hexane (pure)	69	—	100	0·677	30	—
White spirit	150	200	77	0·794	30	—
Non-aromatic kerosene	203	261	99·5	0·789		
Low-aromatic kerosene	168	294	90·5	0·821	30–35	2–3
High-aromatic kerosene	180	260	62	0·845		
White oil (P 31)	300	367	99	0·857	125	31
Medicinal paraffin	320	—	99	0·885	850	300

TABLE 6. *Results of dip tests with lice, using non-aqueous liquids*

Liquid	Immersion time for complete kill			Symptoms observed				Toxic action
	Adult lice	First nymphs	Eggs	Persistence of peristalsis	Opacity of muscles	Time for complete invasion of tracheae: Adult	Time for complete invasion of tracheae: First nymph	
Water	24 hr.	24 hr.	—	1–2 hr.	None	Never	Never	Mechanical
Medicinal paraffin	1 hr.	1 hr.	—	1–2 hr.	None	Never	Never	
White oil (P 31)	20 min.	1 hr.	—	1–2 hr.	None	10–30 min.	Never	
Non-aromatic kerosene	5 min.	1 hr.	—	>6 hr.	None	1 min.	1 min.	
White spirit	15 min.	45 min.	24 hr.	15 min.	Slight	1 min.	1 min.	Mixed
Low-aromatic kerosene	15 min.	1 hr.	—	1 hr.	Very slight	1 min.	1 min.	
High-aromatic kerosene	15 min.	30 min.	—	15 min.	Slight	1 min.	1 min.	
Alcohol	20 min.	20 min.	8 hr.	10 min.	Moderate	(Partial in 1 min.)	Never	Physical
Hexane	5 min.	5 min.	>24 hr.	5 min.	Moderate	1 min.	1 min.	
Xylene	5 min.	5 min.	24 hr.	5 min.	Moderate	1 min.	1 min.	
Trichlorethylene	5 min.	5 min.	2 hr.	5 min.	Pronounced	Never	Never	
Perchlorethylene	5 min.	5 min.	2 hr.	5 min.	Pronounced	Never	Never	
Alcohol 50%, white spirit 50%	5 min.	5 min.	2 hr.	5 min.	Pronounced	1 min.	1 min.	

* Visible if the alcohol is dyed with methylene blue.

in action owing to their aromatic constituents which have some physical action.

Of considerable interest is the synergistic action of a mixture of alcohol and white spirit which is far more toxic than either ingredient alone. This fact was first observed by Hurst (1940) with blowfly larvae, but does not seem to have been recorded before with insect eggs. By dipping louse eggs in mixtures of varying proportions it was found that the most toxic combination approximates to the 50 : 50 mixture (see Table 7).

TABLE 7. *Results of dipping louse eggs in mixtures of alcohol and white spirit*

% of		% kill of eggs by	
White spirit	Alcohol	½ hr. dip	1 hr. dip
10	90	6	—
25	75	54	63
50	50	84	95
75	25	37	71
90	10	25	—

(2*b*) *Dip tests (aqueous solutions)*

(i) *Samples tested*

Lysol (at 2%) has been recommended for treatment of head lice. Formalin was included in the tests to illustrate its low value as a contact insecticide.

(ii) *Results*

Results of dip tests with aqueous solutions are shown in Table 8.

TABLE 8. *Results of dip tests with lice and eggs using aqueous preparations*

Solution	Minimum dipping time for complete kill		
	Adult lice	1st nymphs	Eggs
2% lysol in water	> 1 hr.	> 1 hr.	< 5 min.
10% lysol in water	15 min.	20 min.	< 5 min.
15% formaldehyde in water	> 1 hr.	> 1 hr.	> 1 hr.
25% formaldehyde in water	1 hr.	1 hr.	> 1 hr.
0·3% mercuric chloride in water	> 1 hr.	> 1 hr.	30 min.
1·0% mercuric chloride in water	45 min.	> 1 hr.	15 min.
Quassia infusion in water	< 1 hr.	—	> 1 hr.

TABLE 9. *Results of dust tests with lice and bugs*

Concentration (%)	Substance	% kill of insects		
		Adult lice	Louse eggs	Adult bugs
10	D.D.T.	100*	0	100
5	D.D.T.	100*	—	100
1	D.D.T.	100	—	96
0·5	D.D.T.	61	—	35
0·25	D.D.T.	12	—	—
10	Gammexane	—	0	—
0·1	Gammexane	100*	—	100
0·01	Gammexane	100*	—	25
0·005	Gammexane	90	—	0
0·001	Gammexane	0	—	—
—	AL63	100*	100	83
1	Rotenone (in AL11)	100	0	75
0·5	Rotenone (in AL11)	95	—	—
10	Lauryl thiocyanate	100*	100	100
100	Diphenylamine	100*	100	100
10	Diphenylamine	100	56	83
100	Thiodiphenylamine	40	10	0
100	Dinitroanisole	100*	100	33
10	Dinitroanisole	50	96	—
—	Boric acid	0	0	5
100	Sodium fluoride	0	0	15
100	Mercurous chloride	0	0	15
100	Mercuric chloride	100	—	100
100	Sulphur	15	0	0

100* indicates a complete kill of lice *within* 24 *hr.*

(iii) *Comments*

The high ovicidal powers of Lysol, already noted, are again evident. The eggs are also more susceptible to an aqueous solution of mercuric chloride than the adults and nymphs.

(3) *Dust tests*

(i) *Samples examined*

D.D.T. and Gammexane. Pure crystalline samples of the active principles of these two insecticides were used. They were ground in a mortar and mixed with varying quantities of powdered kaolin as a diluent.

Anti-louse insecticides used during the war. The rotenone-containing substances were tested by Craufurd-Benson & MacLeod (1946) early in the recent war. The AL63 formula was the earliest anti-louse dust in wide use.

A 10% lauryl thiocyanate dust was successfully used by Buxton (unpublished) to reduce lousiness among native labourers in North Africa.

Diphenylamine was the active ingredient (25%) of the dust 'DFA' used to combat lousiness in Moscow during the winters of 1941 and 1942 (Fedder, 1942).

Dinitroanisole is included in the American army louse powder MYL (first formula).

Miscellaneous. Boric acid and sodium fluoride dusts are used against cockroaches. They are said to

have some contact action as well as a stomach poison effect. Thiodiphenylamine was tested because of its analogy with diphenylamine. The results, like those of Ashner & Mager (1945) show that it is decidedly less effective.

(ii) *Results*

The results of these dust tests appear in Table 9.

(iii) *Comments*

The general level of resistance of lice is lower than that of bugs in these tests as it was with the spray method. (It must be remembered, however, that they are not reared or maintained under exactly the same conditions.) The susceptibility of lice to gammexane seems to be extremely high. Gammexane, D.D.T. and derris have apparently little or no ovicidal action on louse eggs. The ovicidal effect of AL 63 must be due to the inclusion of naphthalene. Dinitroanisole, diphenylamine and lauryl thiocyanate have a considerable ovicidal effect though their general insecticidal effect is much less than that of D.D.T. or Gammexane.

Of the miscellaneous group, only the dangerous mercuric chloride is decidedly insecticidal.

Section (B). Insecticidal value of seeds of *Delphinium* spp. (*Ranunculaceae*)

(i) *Samples used*

The seeds of delphinium plants have long been known to have insecticidal powers. Stavesacre or lousebane (*Delphinium staphisagria*) is the source of an ancient remedy for head lice; its advocates extend from Pliny (Nat. Hist. Book 23, chap. 13) to the British Pharmacopoeia 1914 and the B.P. Codex of 1934.

During the recent war, when insecticides were in great demand, the Russians examined their native flora for possible sources of new insecticides. They found that extracts of *Delphinium* and *Aconitum* were among the most powerful of plant insecticides (Petrischeva, 1945). All five of the *Delphinium* species examined gave a positive result, but the most insecticidal were *D. laxiflorum* and *D. elatum* which were said to have a high antiparasitic value, apparently against lice and bed-bugs. On the other hand, the Canadian entomologist Kelsall, who tested the insecticidal powers of *D. brownii*, came to the conclusion that it was not an effective contact insecticide or ovicide as compared with nicotine. He found, however, that delphinium alkaloids were effective as stomach poison insecticides and were powerful repellents (quoted by Holman, 1940).

The active principles in delphinium extracts do not seem to have been definitely established. The seeds contain alkaloids to the extent of 0·5–1% and the following have been isolated by different workers:

Species	Alkaloids	Authors
Delphinium staphisagria	Delphinine, $C_{33}H_{45}O_9N$	Jacobs & Craig (1939)
	Delphisine	Jacobs & Craig (1939)
	Delphinoidine	Jacobs & Craig (1939)
D. ajacis	Ajacine, $C_{34}H_{46}O_9N\ 2H_2O$	Hunter (1943)
	Ajaconine, $C_{22}H_{33}O_3N$	Goodson (1945)
	Ajacinine, $C_{22}H_{37}O_6N$	Goodson (1945)
D. elatum	Delpheline, $C_{22}H_{33}O_5N$	Goodson (1943)
	Delatine, $C_{19}H_{25}O_3N$	Goodson (1943)
D. brownii	Unnamed, $C_{22}H_{37}O_7N$ or $C_{25}H_{37}O_7N$	Manske (1938)

By analogy with other alkaloid-containing vegetable insecticides, one would expect the alkaloids to be the toxic components. Williams (1914) claimed to show that the toxicity of extracts of *D. ajacis* was not in proportion to the amount of alkaloid present, and that a portion of the toxicity was resident in the fixed oil. His methods of testing toxicity, however, seem rather crude.

The tests to be described below were made with extracts kindly prepared by Dr T. A. Henry and Dr T. M. Sharp of the Wellcome Bureau of Scientific Research. Preparations were made from the seeds of five species or varieties of *Delphinium*. Their names will be designated in the tables of results by initial letters, as follows:

Plant	Key letters
Delphinium ajacis L.	*D.a.*
D. ajacis '*florapleno*' (a gardeners' variety)	*D.a.f.*
D. elatum L.	*D.e.*
D. elatum hybridum	*D.e.h.*
D. formosum Bois & Hult *coelestinum* Hort.	*D.f.c.*

Unfortunately it was not possible to include *D. staphisagria* because importation of the seeds of this plant ceased during the war, and they were unobtainable at the time of this investigation.

The extracts were of four types: (i) oil (containing alkaloids); (ii) oil (free of alkaloids); (iii) (oil-soluble) alkaloids; (iv) (water-soluble) alkaloid hydrochlorides.

Mr Goodson of the Wellcome Bureau estimated the following percentages of total alkaloids in some very small samples of some of the Delphinium oils to be as follows: *D.e.h.* 0·98% alkaloids; *D.f.c.* 0·78%; *D.a.f.* 0·65%.

(ii) *Results*

The results of the spray tests are given in Tables 10 and 11. Median lethal concentrations calculated graphically from these results were approximately 5% for the oils containing alkaloids as compared with 50% or more for the alkaloid-free oils. The median lethal concentration of the oil-soluble fraction of the alkaloids extracted from *D.a.f.* was less than ½%.

There was a negligible mortality among louse eggs and also bed-bugs after spraying with any of these oil samples at 50% concentration. However,

TABLE 10. *Results of spray tests with oils containing alkaloids extracted from* Delphinium *seeds. Also tests with alkaloids from one sample*

Concentration sprayed (%)	% kill of lice by: *D.a.f.* oil Sample (i)	*D.a.f.* oil Sample (ii)	*D.a.f.* oil Sample (iii)	*D.e.h.* oil	*D.f.c.* oil	Alkaloids from *D.a.f.* sample (iii)
25	90	100	—	—	—	—
15	79	96	81	90	87	—
7·5	57	52	66	62	67	—
5·0	47	52	67	49	52	—
2·5	—	—	37	—	36	—
1·0	20	—	—	—	29	100
0·5	—	—	—	—	—	80
0·25	—	—	—	—	—	61

TABLE 11. *Results of spray tests with oils from* Delphinium *seeds after removal of the alkaloids*

Concentration sprayed (%)	% kill of lice by: *D.a.*	*D.a.f.*	*D.e.*	*D.e.h.*	*D.f.c.*
50	53	40	42	48	35
25	41	29	22	38	25
15	22	7	18	—	—
7·5	8	7	3	11	6

TABLE 12. *Results of droplet tests with bed-bugs*

Concentration (% in alcohol)	% kill of bed-bugs: *D.a.f.* oil plus alkaloid	*D.a.f.* alkaloid-free fixed oil	Alkaloids
100	94	0	—
50	80	0	—
25	60	—	—
12	20	—	—
2	—	—	60
1	—	—	40
½	—	—	0

TABLE 13. *Results of dip tests with lice (5 min. immersion in aqueous solutions of alkaloid hydrochlorides)*

(Results pooled for *D.a.*, *D.a.f.* and *D.e.*)

Concentration (% in water)	% kill: Adult lice	Eggs
5	100	11
2·5	100	7
1	83	0
0·5	67	—

the bed-bugs showed a temporary paralysis (lasting 2–3 days) after spraying with the alkaloid-containing oils only.

Since the bugs were evidently much more resistant to these substances, an application more drastic than the spray method was devised. Dipping in alcoholic solutions was not feasible, since there was a high mortality among bugs dipped in pure alcohol. Therefore, small droplets (0·4 mg.) of alcoholic solutions were applied to the backs of bugs with a micro-pipette. This rate of dosage applied about ten times as much liquid as would be deposited on a bug by the spray method. The results of these experiments are shown in Table 12. The toxicity of the hydrochlorides of the alkaloids was investigated by dipping lice and their eggs into aqueous solutions of these compounds. The mortalities produced are given in Table 13.

To investigate the possible toxicity of delphinium oils towards mosquitoes, a spray experiment was kindly done by Dr W. A. L. David in a cabinet of his own design (1946), using *Aëdes aegypti*. A 5% solution of *D.a.f.* oil containing alkaloids gave a negligible kill, whereas the American official test insecticide (approximately equal to 0·1% pyrethrins) killed 50% under the same conditions.

(iii) *Comments*

These results prove that the main contact insecticidal power of delphinium extracts resides in the alkaloids. (The mild toxicity of the fixed oils may possibly be due to unremoved traces of alkaloid.) A sign of the intoxication produced by these alkaloids in lice and bugs is more or less complete

paralysis, from which, however, the more resistant bugs are often able to recover. Possibly the lice die from starvation due to inability to feed for several days, a matter which would not inconvenience a bed-bug. An unusual and characteristic sign shown by lice intoxicated by the alkaloids is that the faeces are passed as a continuous dry filament instead of in small pellets.

The poor results with bugs and mosquitoes and the lack of ovicidal power suggests that these *Delphinium* oils are probably not general contact insecticides of the first order. Moreover, the alkaloids themselves are rather highly toxic to man when concentrated, so that they probably will not rival the new synthetic insecticides such as D.D.T. and Gammexane, when and where the latter are readily available.

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Tyroglyphid mites in stored products. Nature and amount of damage to wheat

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The paper describes experiments and observations on the damage done by mites to stored grain, and methods of assessing the damage. It is confirmed that mites cannot penetrate grains if the grain-coat is intact; but usually less than 10% of the grains are so intact.

In laboratory experiments, mites consumed up to 3% by weight of the grain before dying out; they were able to destroy the germ completely, but consumed very little of the remainder; the maximum rate of consumption observed over a 6-week period was 0·29% of grain weight per week; when added to grain at 75% relative humidity in the proportion of 1% by weight, mites consumed 1% of the grain in 27 days at 21° C., or in 37 days at 10–15° C. Although mites consumed the grain more rapidly at higher temperatures or humidities, they survived longer at lower temperatures or humidities, and finally did more damage. Mites consumed wheat germ flakes more rapidly than grain, and finally reduced its weight by 74–85%.

A system of symbols to represent degrees of damage to grain by mites, assessed visually, is presented, and approximate values for the equivalent weight losses are given. The relationship between the product, *no. of mites per 100 c.c. grain* × *time in weeks*, and the degree of damage to grain, was calculated from experimental data; the damage done per unit of this *density* × *time* product was 2–2·5 times higher at 25 than at 5–9° C., and about 1·4 times higher at 70 than at 90% relative humidity. The mean *density* × *time* value corresponding to 1% loss of grain weight was 526,000 at 25° C., and 1,765,000 at 5–9° C.

Data from granary investigations show the degree of damage associated with various levels of population density and grain moisture. In infested storages, the amount of embryo material destroyed ranged from less than 1% of the total (bulk grain) to about 15% (grain in bags).

Mites may cause a rise in the temperature or moisture-content of grain, or an increase in the growth of micro-organisms; but there is no evidence to suggest that serious damage from these causes is likely except that dense infestations can cause heating.

1. Introduction

Methods of assessing the population density of storage mites have been described elsewhere (Solomon, 1945) and also some of the factors limiting their abundance (Solomon, 1946). The present paper deals with the extent and estimation of the damage done by mites to stored wheat.

The observations and experiments described below deal with *Tyroglyphus farinae* L., by far the most important of the mites infesting grain in Britain.

Because tyroglyphids are minute and need small amounts of food, measurement of their food consumption is difficult. Small variations in moisture content cause large errors in estimating consumption, and the numbers of mites cannot easily be determined during an experiment without killing them. At high humidities, fungi develop freely on control samples, while in the experimental samples they provide an extraneous supply of food. Hence the results are generally less precise than in work with larger animals.

In these experiments and in granary investigations (Solomon, 1946), it was found that in reasonably healthy grain the mites confined their attack almost entirely to the germ. The endosperm was consumed only when it was decomposing and unfit for use as human food, owing to the activity of moulds or bacteria under moist conditions (cf. Romanova, 1936).

The importance attached to the loss of the germ may depend upon its value as a by-product of milling, or, if the germ is to be included in the flour, on its vitamin content.

Contractions used: M.C. for 'moisture content' (% of total weight); R.H. for 'relative humidity' %.

2. Resistance of intact grains to mite attack

It seems that seeds and grains are not susceptible to attack by tyroglyphids unless they are damaged mechanically or by disease, and several workers have reported that undamaged wheat is immune (see Solomon, 1943). However, as most wheat grains have the testa abraded or cracked near the germ end, mites are able to penetrate it and attack the germ. In Manitoba wheat, usually less than 10% of the grains have the grain-coat near the germ intact.

These undamaged grains survive with complete embryos even when the mites have completely consumed the embryos of the other grains.

In experiments in which *Tyroglyphus* was reared on small quantities of Manitoba grain at 75% R.H., temperatures 21 and 9° C., 2·1–9·5% of the grains remained undamaged by the mites. Numbers of mites were kept in tubes with grains with the coat intact. The mites died without doing any damage to the grain, although controls on grain with cracked coats penetrated successfully to the germs and ultimately destroyed them.

3. Amounts of grain consumed in laboratory cultures

Description of experiments

Measured numbers (or weights) of mites were cultured on measured quantities of grain, under controlled physical conditions, and the amounts of grain consumed were determined by weighing. Manitoba grain was kept in wire gauze baskets in a desiccator with a KOH solution to maintain the required humidity. The desiccator was fitted with an internal fan. The baskets of grain were left to equilibrate in the desiccator for several weeks if necessary, until their weight reached a fairly steady level. Then the grain was divided into 5 or 10 g. aliquots and transferred to the experimental tubes.

These were usually flat-bottomed 3 × 1 in. specimen tubes; much smaller tubes were used for single grains. They were fitted with fairly tight cotton-wool plugs, coloured so that mites could be easily seen. They were kept in a desiccator containing the appropriate KOH solution. After the grain was transferred to these tubes, they were weighed on a fine balance at intervals of a few days, until no further continuous increase or decrease occurred. The weights never became absolutely steady, probably because of temperature variations. The experiments at 21° C. were conducted in a controlled-temperature room where the variation was about ± 1°. Those at lower temperatures were made in an underground room where the temperature changed gradually, but fluctuated very little. In addition to the weight check, the M.C. of the grain was estimated at the beginning and end of each experiment by grinding the grain from some of the tubes and drying at 115° C. for 4½ hr. in a hot-air oven fitted with a fan.

The mites were cultured on wheat germ flakes in small jam jars, kept in desiccators with KOH solutions to maintain a humidity of 75%. The jam jars were fitted with corks or with press-on metal lids with rubber rings, each lid or cork having a large hole covered with bolting silk or plugged with cotton-wool. For some experiments, small numbers of adult and late nymphal mites were counted out, but for most a quantity of mites in all stages of development was weighed out. The mites were added to the experimental tubes containing moisture-equilibrated grain. In all trials a few experimental tubes were left free of mites, as controls.

At intervals during the course of each experiment, some of the tubes were taken from the desiccator, the mites separated from the grain by thorough sieving, and weighed or counted. The grain was also weighed. Then mites and grain were returned to the tube and desiccator.

The conditions and main results of Exps. 1–5 are given in Table 1.

Exp. 6. Temp. 21° C. A number of approx. 10 g. aliquots of grain, suitably equilibrated, were kept in plugged tubes in desiccators at four humidities, 60, 67, 73 and 82% R.H. At each humidity mites were added to two of the tubes at the rate of 50 mites/100 c.c. grain, to two at the rate of 150 mites/100 c.c. grain, to two at 1500 mites/100 c.c., and to two at 40,000 mites/100 c.c. (≒0·2% by weight); several tubes were left without mites, as controls. The mites were removed at long intervals and returned when the grain had been weighed.

Under the experimental conditions the mites did not survive at 60% R.H.; at 82% R.H. the cultures were spoiled by heavy mould growth. The net reductions in weight in the grain at 67 and 73% R.H. are shown in Table 2.

Exp. 7. Temp. 21° C., R.H. 77%. Wheat-germ flakes were sieved over metal gauze (24 meshes/in.), so that smaller pieces were eliminated. After moisture equilibration, about 100 mg. of flakes were added to each of eight tubes; to six of these about 33 mg. of mites were added. (33% mites on pure germ would be equivalent to 0·66% mites on grain. The germ flakes included a proportion of branny material. However, the proportion of mites to germ was of the same order as in Exps. 1–5.) The two controls became mouldy, so that the results given below are approximate values only.

After 53 days, the loss in weight of the wheat germ was 70·5%. The mites from each tube were replaced by a fresh lot. After a further 17 days (total 70), the total weight loss was 73·9%, and very few mites remained alive.

Exp. 8. Temp. 21° C., R.H. 65%. Except for the lower humidity, this was a replicate of Exp. 7, but the same lot of mites was returned to each tube after weighing throughout.

The loss in weight (corrected by controls) was as follows:

Time in days	9	17	26	31	44	52
Net wt. loss %	14·3	21·9	27·9	31·8	41·2	47·1
Time in days	60	68	95	122	186	
Net wt. loss %	50·8	53·8	73·1	84·2	85·2	

TABLE 1. *Consumption of grain by mites in laboratory cultures*

Exp. no.	Temp. °C.	R.H. %	No. of replicates	Grains no. or wt.	Rate of infestation	Duration of infestation (days) Original	Duration of infestation (days) Subsequent*	Survival of mites	Consumption of grain (% by wt.)
1	8–15	75	7	1 grain	40 mites/grain (≒0·4 % of grain wt.)	133	—	All dead in 19 weeks except on 1 grain	1·72–3·25
2*a*	21	65	3	1 g. of half-grains (beard ends)	3000 mites/portion of grain (≒1 % by wt.)	29		All dead	0·21
							18		No additional
2*b*	21	65	3	1 g. of half-grains (germ ends)	Do.	29		Some living	0·98
							18		0·98 additional
3*a*	21	75	3	1 g. of half-grains (beard ends)	Do.	23		A few living	0·10
							18		0·01 additional
3*b*	21	75	3	1 g. of half-grains (germ ends)	Do.	23		A few living	1·09
							18		0·60 additional
4	21	75	5	5 g.	1 % by wt.	82		Almost all dead	2·11
							7		0·22 additional
							11		No additional
5	10–15	75	4	5 g.	1 % by wt.	84		Some living	2·18
							8		No additional

* At the end of the first trial, any survivors were removed and a second lot of mites added.

TABLE 2. *Data from Exp.* 6. *Percentage reductions in weight of approx.* 10 *g. lots of grain caused in various periods by mites at four different (original) densities, at* 67 *and* 73 % R.H.

Original nos. of mites per 100 c.c. grain	Loss in weight of grain after various periods from start (%) 8·5 weeks (60 days) 67 % R.H.	8·5 weeks (60 days) 73 % R.H.	26 weeks (182 days) 67 % R.H.	26 weeks (182 days) 73 % R.H.	44 weeks (308 days) 67 % R.H.	44 weeks (308 days) 73 % R.H.
50	0·04	0·27	0·52	1·11	2·18	1·90
150	0·05	0·14	0·50	1·87	1·98	2·40
1,500	0·10	0·42	1·54	2·11	2·66	2·71
40,000	0·57	1·04	2·47	2·20	2·85	2·78

All the mites were dead at 186 days. The weight loss at 26 days was equal to the original weight of the mites.

Many difficulties were encountered in the course of the experiments. Some of the tubes of grain, particularly controls, were spoilt by heavy mould growth. Although the food materials were brought to a steady weight before the mites were added, they underwent subsequent variations in moisture content, possibly owing to temperature variations. On the whole, the mites developed less rapidly and successfully than expected. Hence, the values given for the amounts of materials consumed, and more particularly the values given below for the rates of consumption cannot be regarded as exact, and are likely to be under-estimates. With these reservations, certain conclusions may be drawn, as below.

(*a*) *Consumption of endosperm.* The amount of germ material in the grain used was about 2 % by weight.† In Exp. 1, up to 3·25 % of the weight of single grains was lost. In Exp. 6, 2·85 % of the weight of a quantity of grain was consumed at 67 % R.H., and 2·78 % at 73 % R.H. Hence, under the conditions of the experiments, a limited quantity of endosperm was consumed, in addition to the germ. In Exps. 2 and 3, at 65 and 75 % R.H., mites consumed only 0·21 and 0·11 % respectively of cut grains deprived of their germs, i.e. mites consumed only a small quantity of the endosperm.

† Determined by dissecting the germ from ten grains of various sizes and weighing on a fine balance. Values ranged from 1·7 to 2·3 %.

(*b*) *Maximum amounts of grain consumed.* The data in Table 3 show that, under the more favourable experimental conditions, mite populations generally consumed from 2 to 3 % by weight of the grain before dying.

(*c*) *Rate of consumption of grain.* Generally, the rate of consumption of grain was higher in the early stages of each experiment and fell gradually as the mites died. Taking the mean weekly consumption over the first 6 weeks, the following are the highest figures obtained (Exp. 1 is omitted, since there were no observations in the early stages):

Exp. 4 (21° C., 75 % R.H.): 0·29 % per week.

Exp. 5 (10–15° C., 75 % R.H.): 0·18 % per week.

TABLE 3. *Greatest amounts of grain consumed by the mite populations before dying*

Temp. ° C.	R.H. %	Wt. consumed %	Time (days)	Remarks
8–15	75	3·25	168	Five of the
		2·87	122	7 grains in
		2·81	122	Exp. 1
		2·56	136	
		2·49	122	
10–15	75	2·18	84	Exp. 5
21	67	2·85	308	Exp. 6
	73	2·78	308	
21	75	2·33	89	Exp. 4. Two lots of mites

The time taken by mites to consume 1 % by weight of the grain ranged from 27 days upwards. The shorter times are shown in Table 4.

TABLE 4. *Minimum times taken by mite populations to consume 1 % by weight of grain*

Exp.	Temp. ° C.	R.H. %	Original wt. mites as % grain wt.	Time to consume 1 % of grain (days)
4	21	75	1	27
5	10–15	75	1	37
6	21	73	0·2	55–60
6	21	73 and 67	See Table 2	

(*d*) *Influence of initial population density on consumption.* In most of the experiments the numbers of mites soon fell, so that considerable difference existed between original density and that in the later stages of the experiment. Nevertheless, Exp. 6 (Table 2) showed that the effects of initial concentration differences, while gradually decreasing, persisted throughout the 44 weeks of the experiment. In all cases, owing chiefly to the heavy mortality, the difference in the amount of grain consumed was less than the difference in initial concentration of mites.

(*e*) *Influence of temperature on consumption.* Some aspects of the influence of temperature may be seen by comparing Exps. 4 and 5, both at 75 % R.H.:

	Exp. 4	Exp. 5
Temperature (° C.)	21	10–15
Time to consume 1 % of grain (days)	27	37
Amount consumed per week (1st 6 weeks only) (%) ...	0·29	0·18
Total amount consumed (approx. 12 weeks) (%) ...	2·11	2·18

The rate of consumption was at first higher at the higher temperature, but the final total consumption was about the same at both temperatures.

(*f*) *Influence of humidity on consumption.* In Exp. 6 (Table 2) in the earlier stages, the mites consumed more grain at 73 than at 67 %, at all four concentrations. But after a long period there was little difference between the amount consumed at the two humidities. Similarly, in Exps. 2 and 3 the half-grains containing germ were consumed more rapidly at first at the higher R.H. (75 %), but ultimately a greater quantity was consumed at the lower R.H. (65 %). Again, in Exps. 7 and 8, the wheat-germ flakes were consumed more rapidly at the higher R.H. (77 %), but the mites ultimately consumed more at the lower R.H. (65 %) at which they survived for a much longer period.

These data show that while high humidity at first favoured rapid consumption of the food, other factors gradually intervened, so that the mites survived longer, and ultimately consumed more food, at humidities below 70 % R.H. This suggests that in these experiments some actively unfavourable factor, such as the growth of pathogenic micro-organisms, or an accumulation of carbon dioxide, must have operated at the higher humidities.

(*g*) *Consumption of flaked wheat germ.* The time taken to consume 70 % by weight of this material was 13 weeks at 65 % R.H. (Exp. 8), and only 7·5 weeks at 77 % R.H. (Exp. 7). But at 77 % R.H. the mites died out in 10 weeks, having consumed 73·9 % of the material, whereas at 65 % R.H. they survived for 18–26 weeks and consumed 85·2 %.

4. A SYSTEM OF DEGREES OF GRAIN DAMAGE

The damage done to grain by mites is readily recognizable because of their characteristic mode of attack. Grains attacked by mites usually have a small hole in the grain-coat near the tip of the embryo, which may be completely hollowed out, leaving a cavity still largely covered over by the grain-coat. Certain insects also attack the embryo, but they do not leave behind the small pieces and debris usually left by mites, and they remove more of the grain-coat overlying the germ cavity.

The proportion of the grains in which the embryo has been attacked, and the proportion of the grains in which the embryo has been completely destroyed can readily be determined by microscopic examination. On this basis, a system of degrees of damage can be set up, as in Table 5.

This system, using the symbols *a*, *b*, *c*, *d*, *e*, *f*, has been used in the work described in this and an earlier paper (Solomon, 1946). It is more satisfactory, however, to use the letters and numbers shown in columns 2 and 3 of Table 5, e.g. C2 instead of *d*. When this is done, unusual combinations, such as D2, can be indicated as readily as the common ones.

TABLE 5

Degree of damage	Proportion of grains with damaged embryos	Proportion of grains with embryos completely destroyed
a	None attacked	(1) No embryos completely destroyed
b	A minority attacked (B)	(1) No embryos completely destroyed
c	A majority attacked (C)	(1) No embryos completely destroyed
d	A majority attacked (C)	(2) A minority of embryos completely destroyed
e	Over 90% attacked (D)	(3) A majority of embryos completely destroyed
f	Over 90% attacked (D)	(4) Over 90% of embryos completely destroyed

TABLE 6. *Approximate limits of values for percentage weight loss, in grain damaged to the extent indicated by the symbols. The weight loss in large numbers of grains taken together approximates to the mean values*

Symbol for degree of damage	Weight loss, as % original total weight			
	Germ only		Whole grain	
	Min. and max. value	Mean	Min. and max. value	Mean
a	0	0	0	0
b	Up to 10	5	Up to 0·2	0·1
c	10–20	15	0·2–0·4	0·3
d	20–65	42	0·4–1·3	0·84
e	65–90	77	1·3–1·8	1·54
f	Over 90	92	Over 1·8	1·84

5. Degrees of damage in terms of weight loss

In the experiments described in §3, observations were made of the grain damage according to the above system. Simultaneous records of the weight lost by the mite-infested grain were available. On this basis, estimates were made of the relationship between the visual damage and the weight loss. Table 6 shows the typical limiting and mean values for weight loss, associated with each degree of visual damage.

The maximum and minimum values shown give only an approximate indication of the usual limits. The mean germ weight is taken to be 2% of the total grain weight.

6. Degree of damage in relation to population density and exposure time

A useful means of assessing the destructive effects of pests is to examine the relationships between the damage done and the product, *population density* × *time*. In the experiments in §3, the only record of the changes in population density was provided by sifting the mites away from the grain at intervals, and weighing them. This proved unsatisfactory, because of the quantity of dead bodies and other debris which could not be separated from the living mites.

In another series of experiments, the numbers of mites were estimated at intervals by counting. Since the method adopted involved immersing the mites in oil (Solomon, 1945) they could not be returned to the experiment. Therefore a number of replicates of each culture tube were used. The degree of damage to the grain was visually estimated at the same time. n most other respects, the experiments were similar to those described in §3. Specimen tubes of grain, plugged with cotton-wool, were kept in desiccators containing standard humidity solutions of KOH. The 4 × 1 in. tubes each contained approx. 20 g. of grain which had been brought to moisture equilibrium with the experimental humidity. A weighed quantity of *Tyroglyphus* (approx. 15,000 mites, all stages) was added to each tube. Sets of tubes were kept at 25° and 5–9° C., at 90 and 70% R.H., making four sets of 8–10 tubes.

Each experiment continued for 10–14 weeks, and at approx. 2-weekly intervals during this period, pairs of tubes were taken out for examination. (Half the tubes contained numbers of the predator, *Cheyletus eruditus* Schr. For each examination, one of these tubes was taken out, and one containing only *Tyroglyphus*. For the present purposes, the relevant point is the number of *Tyroglyphus*, whether or not *Cheyletus* was present.) The mites were then separated from the grain by thorough sieving, and counted, while the grain was examined under a low-power binocular microscope, for assessment of the damage due to mites.

Since the experimental tubes had to be discarded after examination, different tubes were examined at each time-interval. For the purposes of calculation, however, the successive population figures were treated as stages in the increase or decrease of a single population. The mean of the population figures (numbers of mites per 100 c.c. grain) at the beginning and end of each time-interval was taken as the population density for that period, and was multiplied by the time in weeks. At the end of the second period, the products for the first and second

periods were added together, and so on. The values of the product corresponding to different degrees of grain damage, under various physical conditions, are shown in Table 7. The following points emerge from an examination of the data:

(*a*) At each temperature and R.H. there is a wide range of values of the *density* × *time* product. A fairly wide range is to be expected, since each degree of damage is a broad category. However, at the same temperature and R.H., there is in some cases considerable overlapping between the ranges of values of the product corresponding to successive degrees of damage.

therefore in the rate of consumption of food. (Measurements of the rate of carbon dioxide production by mites in grain, undertaken to test this point, confirmed that the metabolic rate is considerably higher at 90 than at 70% R.H.)

It is probable that the mortality at the lower humidity is comparatively high. This would cause an increase in the apparent rate of damage per unit of density, because of the mites which would have died after contributing to the damage, but before being counted. A further possibility is that, because of the greater rate of water loss by the mites at the lower humidity, extra food is eaten, simply as a

TABLE 7. *Values of the product of the population density × time, in relation to the damage done to grain by* Tyroglyphus *in experimental cultures, under the temperature and humidity conditions shown. Density in no. of mites per* 100 *c.c. grain; time in weeks*

Damage ...	*b*		*c*		*d*		*e*		*f*
R.H. % ...	70	90	70	90	70	90	70	90	90
Sat. def. (mm. Hg) ...	1·96–2·76	0·65–0·92	1·96–2·76	0·65–0·92	1·96–2·76	0·65–0·92	1·96–2·76	0·65–0·92	0·65–0·92
Temp. 5–9° C.	99,000	267,000	294,000	117,000	—	864,000	—	—	—
	102,000	312,000	367,000	165,000	—	886,000	—	—	—
	204,000	—	374,000	465,000	—	1,643,000	—	—	—
	223,000	—	381,000	470,000	—	—	—	—	—
	316,000	—	616,000	1,556,000	—	—	—	—	—
Mean	189,000	290,000	406,000	555,000	—	1,131,000	—	—	—
	$p < 0·1$		$p < 0·6$						
Sat. def. (mm. Hg) ...	7·13	2·38	7·13	2·38	7·13	2·38	7·13	2·38	2·38
Temp. 25° C.	—	—	133,000	191,000	196,000	312,000	799,000	573,000	853,000
			152,000	322,000	238,000	340,000	818,000	—	—
			358,000	—	244,000	483,000	—	—	—
Mean	—	—	214,000	257,000	266,000	378,000	809,000	573,000	853,000
			$p < 0·8$		$p < 0·05$				
Significance of differences at two temperatures			$p < 0·1$	$p < 0·4$		$p < 0·05$			

(*b*) Omitting cases where there are not at least two figures at each R.H., there is a higher mean value of the product at 90 than at 70% R.H., for each degree of damage, both at 5–9 and at 25° C. The mean ratio of the products is approximately the same at both temperatures, namely, 0·7 (at 70% R.H.): 1·0 (at 90% R.H.), i.e. the mites caused about 1·4 times as much damage to the grain, per unit of *density* × *time*, at 70 as at 90% R.H. While the individual differences are not statistically significant, taken all together they strongly suggest that the differences arise from a real tendency.

Such a result was not expected, for the rate of multiplication is normally much higher at 90 than at 70% R.H.; figures given by Zakhvatkin (1941) show considerable differences between the rate at 80 and that at 90% R.H. It is reasonable to infer corresponding differences in the metabolic rate, and source of moisture. This would further complicate the relationship between the metabolic rate and the rate of damage per unit of density.

(*c*) There are three columns in Table 9 with sufficient data for an examination of the effects of temperature on the value of the *density* × *time* product corresponding to a certain degree of damage at one R.H. These are *c*, 70%; *c*, 90%; and *d*, 90% R.H. The bottom line of the Table shows the significance of the differences between the means. Taking the three pairs together, the difference between the figures at the two temperatures is too marked to be easily dismissed. The ratio (mean of the three pairs) of the values of the product is approx. 0·44 (at 25° C.): 1·0 (at 5–9° C.), i.e. the mites caused 2 or 2·5 times as much damage at 25° as at the lower temperature, per unit of *density* × *time* product.

The ratio of the rates of multiplication at these

two temperatures, according to Zakhvatkin (1941) is at least 7 : 1. If this figure is not too high an index of the effect of temperature on the metabolic rate, conditions in the above experiments must have been unfavourable enough to cause a marked reduction in the usual difference due to temperature. However, it is possible that the net rate of multiplication at different temperatures is not a reliable index of the metabolic rate and the rate of feeding, because of effects of temperature on the mortality rate.

(*d*) The data gave no indication that the results were affected to an important extent by overcrowding. The mean population density from the beginning of the experiment to the time of observation ranged from 19,800 to 144,200 mites per 100 c.c., i.e. about 8–60 mites per grain. In a few of the tubes, the predators had reduced the mean population density to a little more than 25% of that in the corresponding tubes without predators, but no markedly aberrant results could be traced to this source.

The grain at 25° C., 90% R.H., became visibly mouldy at an early stage, but the resulting data did not differ clearly from the other results. It was thought that the presence of mould might reduce the apparent rate of damage, by providing an extra supply of food, or that it might cause an increase in the rate of destruction of the germ, by making it less resistant to attack. However, there was no marked tendency in either direction. More data would be required for a study of this point.

(*e*) In the earlier stages of the experiments in § 3, the rate of consumption of food was higher at 73–77 than at 65–67% R.H. Similarly, in the present series, the rate of consumption was higher at 90 than at 70% R.H., since the denser populations at the higher humidity more than compensated for the lower rate of consumption per unit of density.

In the later stages of the experiments in § 3, there was a falling off in the population at the higher R.H., and the mites died before eating as much of the grain as was ultimately consumed at the lower humidity. There was no evidence of this in the present experiments.

(*f*) The approximate weight losses corresponding to each degree of grain damage have been given in Table 6. Using the mean values, and the data in Table 7, the *density* × *time* values corresponding to 1% loss of grain weight were calculated. The resulting mean values in terms of *number of mites per* 100 *c.c. grain* × *time in weeks* are 526,000 (standard deviation 272,000) at 25° C., and 1,765,000 (standard deviation 1,120,000) at 5–9° C.

7. Degree of damage to stored grain in relation to population density and moisture content

From the study of a large number of samples of infested Manitoba grain, taken from storages both in bulk and in bags, it has been shown elsewhere (Solomon, 1946) that there is a considerable degree of correlation between the M.C. of the grain and the population density of the mites. The majority of these samples were also examined to find the degree of damage to the grain.

Damage by mites is related to the population density which is in turn related to the M.C. A very close relationship between damage and population density in samples is not to be expected, since the damage is due to the past history of the infestation as well as to the current population. In some of the samples containing few or no living *Tyroglyphus* the characteristic damage to the grain was, nevertheless, recognizable, and such samples contained more or less copious remains of dead mites.

Any relationship between damage and M.C. is chiefly indirect, through the influence of M.C. on the population density. Hence it is subject to the disturbances mentioned above, and is also affected by factors which complicate the relationship between M.C. and population density, such as the activities of the predatory mite, *Cheyletus eruditus* Schr., temperature variations, and variations in the proximity to other infested grain.

Nevertheless, the data showed that each degree of damage was associated with a range of population density and of M.C. which was characteristic at least in its upper limits (Table 8). Owing to the factors mentioned, little significance can be attached to the lower limits of each range.

Table 8. *Maximum population density and* M.C. *values associated with each degree of damage in* 300 *samples of grain infested, or previously infested, by* Tyroglyphus

Degree of damage	Maximum population density (mites per 100 c.c. grain)	Maximum moisture content of grain %
a	20 (50 in one case)	14·0
b	1,600	15·8
c	10,500	15·5
d	31,000	18·4
e	100,000	17·4
f	250,000	25·0

Table 9. *Minimum damage to grain infested by* Tyroglyphus, *at successive levels of population density. Based on* 115 *samples*

Population density (no. of mites per 100 c.c. grain)	Minimum damage to grain
0–20	*a*
20–2,000	*b* (*a* in one case)
2,000–10,000	*c*
10,000–30,000	*d* (*c* in one case)
30,000–100,000	*e* (*d* in one case)
Over 100,000	*f*

Table 9 shows the degrees of damage associated with various levels of population density. In this case the higher values for the damage at each level are of little importance because they arise from secondary reductions in population density, but the minimum values are significant.

8. Extent of damage in grain stores

It is seldom that all the grain in a storage place is uniformly moist and infested. The initial M.C. of Manitoba wheat is usually too low to support large numbers of *Tyroglyphus*. But in English storage conditions, peripheral parts of a bulk take up extra moisture from the air and sometimes from damp floors and walls, so becoming more susceptible to infestation (Solomon, 1946). The intensity of infestation usually corresponds to gradients of M.C. The following examples give some idea of the resultant damage in relation to the bulk as a whole. The damage was estimated by examining numbers of samples.

(*a*) *A granary in London.* A bulk of several hundred tons on the ground floor was in contact with the brick walls. About half the wall on one side was exposed to the weather, and had passed moisture through to the grain. The depth of the bulk near this wall was about 60 cm. The M.C. was 11·8% or less in the depths of the bulk, about 13% at the surface, and 14·5–15% a few cm. from the exposed wall. The mite damage extended, on an average, about 15 cm. inwards from the wall. There was a layer of grain, in which more than half the germ had been destroyed, extending about 4 cm. from this wall. This infestation was not more than 4–5 months old.

(*b*) *A granary in Gloucestershire.* This was a 6-storey brick building. About 6000 tons of bulk grain was stored on the five upper floors each measuring 44 × 13 m., with the grain 1–2 m. deep. In many places, the walls were damp and the adjacent grain had become infested by *Tyroglyphus*. In August 1940, the grain had been 15 months in storage, and it seemed probable that the mite infestation was 9–12 months old. The M.C. in the depths of each bulk was about 11·5%, but higher towards the periphery. The grain within a metre of the damp walls showed a sharp M.C. gradient, rising towards the wall to 14–20% M.C. or even higher, and was generally mite-infested. The grain showing mite damage extended on an average 50 cm. from the wall, while grain in which more than half the embryo was destroyed extended about 15 cm. from the wall. Approximately 1% of the total bulk was mite-damaged, and 0·3% of the total bulk had at least half the germ material destroyed. During the following 12 months, there was no marked extension of either moisture or mites.

(*c*) *A store in Devon.* About 300 tons of grain, stored in bags, stood vertically on the concrete and brick floor at ground level, partly on wooden dunnage and partly on straw. The stack was 3 bags high. In July 1941, the wheat had been in this store about 8 months. The M.C. was 13·0–13·1% in the less exposed grain, rising commonly to 13·4%, occasionally to 16 and even 19%. It was infested throughout by *Tyroglyphus*. About 15% of the total embryo material had been destroyed by the mites.

(*d*) *A store in Yorkshire.* A large parcel of wheat was stored in bags stacked three high, vertically. The grain stood on a layer of straw over a single layer of tarpaulins covering a concrete floor at ground level. The wheat had been stored here for 2½–3 years. The M.C. of the innermost grain was 12·8–13·0%, increasing towards the sides and tops of the stacks, and more particularly towards the bottom, where moisture had come through the concrete and tarpaulins, to 14–20%, and higher in some places. Only the lowest tier of bags was generally infested, but much of this grain was severely damaged. About 11% of the embryo material of the total bulk (all three tiers) was destroyed.

9. Indirect damage to grain

It has often been stated that mites cause indirect damage to grain by raising its temperature, or its M.C., or by encouraging the growth of micro-organisms. It seems inevitable that all three phenomena should occur, but apparently no observations have ever been made to determine the extent of these effects, and whether they have any practical significance.

(*a*) *Heating.* Rathbone (1919) considered that the presence of mites could initiate serious heating of stored grain; Voloschuk (1936) also stated that mites contributed to heating, while Zacher (1927) denied it.

Howe & Oxley (1944) measured the concentration of carbon dioxide produced by various pests in 24 hr. at 25° C. in a jar full of grain. A 1% 'CO_2 figure' was produced by 0·15 g. *Tyroglyphus* per pound of grain (≡8000 mites per 100 c.c. grain). Oxley & Howe (1944) calculated the corresponding heat production, and concluded that a 1% 'CO_2 figure' would not involve a significant rise in grain temperature unless a very large bulk, such as 6 m. cube, were infested throughout at this density. But a CO_2 figure of 10–20% would involve a considerable temperature rise in a bulk of grain only 1 m. cube. The mite density necessary to produce such a CO_2 figure would be 80,000–160,000 mites per 100 c.c. grain, a level which is not uncommon, but is not usually attained by large sections of a bulk.

Thus mite infestation may cause serious heating of grain. In imported bulk grain, however, any mite infestation is usually restricted to a portion, of which

there is usually only a small fraction carrying dense populations (cf. § 8). Hence the heating effects due to mites are likely to be much more restricted and less dangerous than those due to certain insect pests.

(*b*) *Moisture.* Tyroglyphids are attracted to moist materials, and tend to develop more rapidly as the moisture increases (see Solomon, 1943). But there are no published data showing the extent of the converse effect—an increase in grain moisture due to mites. Considerable local increases in M.C. may occur as the result of transference of moisture away from heating grain (Oxley & Howe, 1944) and mites may certainly be responsible for the heating.

Oxley & Howe (1944) pointed out that the rate of production of metabolic water by pests is too low to cause any spectacular increases. They calculated that even a relatively heavy infestation, producing a 'CO_2 figure' of 7% (≡56,000 mites per 100 c.c. grain) would take about a year to raise the moisture content of the grain by 1%, even if no water were lost. Mite populations much denser than this cannot maintain themselves for long periods on the same grain, as the grain embryos are rapidly removed, and the resulting food shortage leads to the death or dispersal of the population.

In the course of experiments, such as those described in § 3, there have been many opportunities of detecting any large moisture increases due to mites, but no instances of this have been observed.

(*c*) *Micro-organisms.* Rodionov (1940) stated that severe infestation of grain is usually accompanied by a mass development of micro-organisms, especially fungi, which the mites disseminate. Sigrianskii (1940) showed that mites in grain disseminate some of the common fungal diseases of the wheat plant. Beattie (1918) reported briefly on the bacteria found in samples of flour, some of them mite-infested, and more recently Dr S. E. Jacobs, of the Imperial College of Science and Technology, has studied the micro-organisms in mite-infested grain (unpublished work). He found that the bacterial flora of Manitoba wheat changed, as its M.C. increased, from one of Gram-negative rods to one of Gram-positive cocci. This change was slow at 15·4% M.C. but very rapid at 18·6%. When mites were present the change was accelerated, and the numbers of bacteria increased markedly, especially at the higher M.C. Fungi developed in the moister mite-free grain. Masses of conidia formed at 18·6% M.C. in the absence of mites, but were largely absent in mite-infested grain, although the fungal count was still relatively high.

It is noticeable that in experimental tubes of grain at fairly high humidities, the mites often hinder the growth of moulds to a marked degree, by feeding on them. For this reason, control tubes without mites are often damaged by heavy mould growth, long before the mite-infested grain is seriously affected. Schulze (1924) observed that females of *Caloglyphus rodionovi* Zakhv. kept their food materials free of moulds; she attributed this to the action of an alkaline secretion produced by the mites.

(*d*) *Taint.* The pungent 'minty' odour of *Tyroglyphus farinae* is easily detectable in heavily infested materials, and other species have characteristic odours (see Solomon, 1943, 1944). The 'minty' smell of *Tyroglyphus* is associated with a lipoid material which can be extracted by immersing the mites in ether. This lipoid probably originates from the paired dorsal 'oil glands' and spreads over the cuticle.

In some materials taint may be a serious matter, but it is probably effectively removed from grain, together with most of the mites, in the cleaning and washing processes before milling.

The experimental work was performed, under direction, by Miss A. Phillips. I am indebted to Dr S. E. Jacobs, of the Imperial College of Science and Technology, for permission to quote results of his unpublished work. Mr R. W. Howe assisted with advice on statistics. The work forms part of the programme of the Pest Infestation Laboratory, and is published by permission of the Department of Scientific and Industrial Research.

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Study of an infestation by *Laemophloeus* sp. (Coleoptera Cucujidae) in bulk wheat

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(With 1 Text-figure)

Physical and biological data are presented from an infestation by *Laemophloeus* sp. (unidentified but very near to *L. minutus* Oliv.) in bulk wheat contained in open-topped silo bins. Much of the life history of this species is spent within the grains of wheat under the conditions of this infestation. It follows that the density of population of this species in whole wheat can be estimated only by breeding out or by determining the carbon dioxide output of the grain. Some larvae are free-living for part of their lives. No regular relation has been found between the number of larvae free-living at any time and the number of insects remaining within the grains. The proportion of the total population which is free-living is least when the total population is greatest. There is some evidence that the grain contained a light infestation of *Laemophloeus*, fairly evenly distributed, before it was loaded into the silo bin. It is supposed that this was the source of the infestation studied. Factors which might be responsible for the uneven development of the population are discussed.

INTRODUCTION

Species of *Laemophloeus* occur widely as pests of stored grain but usually only in small numbers and apparently causing little damage. The species of this genus which attack stored grain are usually described as secondary pests (e.g. Linsley & Michelbacher, 1943), i.e. pests which cannot or do not attack undamaged grain and can therefore only infest grain which has already been attacked by a primary pest. Until the past few years there has been no evidence to contradict this view. In the later years of the war, however, examples became more common in Britain of infestations in which this insect was present in very large numbers indeed, causing considerable heating and damage, while primary pests, though usually present, were so sparse as to be insignificant. During the same period, in Canada, infestations occurred in which *Laemophloeus* was either the primary pest or at least the dominant pest in bulk wheat and caused considerable damage (Smallman, 1944).

THE CIRCUMSTANCES OF THE INFESTATION

In November 1944 an opportunity occurred to examine a *Laemophloeus* infestation associated with heating in Canadian 'Manitoba' wheat stored in silos at Birkenhead. This was the first occasion on which it had been possible to study such an infestation in a silo with apparatus capable of determining temperatures and of sampling the grain and its intergranular atmosphere at all depths. Unfortunately, by the time of the inspection, the most heavily infested wheat had been removed and there remained only three bins which were sufficiently infested to warrant study. Bin 21, loaded in March 1944, was sampled at all depths and only data from

this bin are presented here. The other bins were sampled more superficially and data from these were in agreement with those from bin 21. Each bin contained wheat from a different shipment.

The silo consisted of square-section, open-topped concrete bins, approximately 12 ft. (3·7 m.) square by 65 ft. (19·9 m.) deep, built within the outer walls of an old, floor-type warehouse from which the floors had been removed. The building was covered by a corrugated iron span roof with access by wooden gangways built over the top of the bins. Loading was from open-band conveyors running along each gangway.

Apparatus and methods

Deep sampling of the grain was carried out by a special apparatus capable of collecting samples, each of 200 g., in vertical series at intervals of 1 m. depth.* From each sample of grain a 25 g. subsample was immediately removed for subsequent determination of water content. The remainder of each sample was transferred to a tin and tightly covered with muslin. The samples were examined at the laboratory in Hull on the second day after they were taken. The procedure for examination of the samples was as follows:

(1) Free-living insects in each sample were counted; living and dead adults and dead larvae were removed; living larvae were left in the sample. All dust was returned to the sample.

(2) Each sample was kept for 6 weeks in an open-topped glass jar covered with muslin and maintained at 28° C. and 70% R.H. Every week the initial examination described above was repeated. This process of 'breeding out' was carried out to estimate the numbers and ages of immature insects not visible at the first examination.

(3) Eleven of the samples were selected for determination of their 'carbon dioxide figure' according to the method of Howe & Oxley (1944), after removal of the adults. Incubation for this purpose was at 28° C. for 24 hr., starting 48 hr. after removal of the samples from the bin.

Temperatures were taken by means of long thermocouple ropes, inserted by temporary attachment to metal rods screwed end to end, and a potentiometer of the type described by Oxley & Henderson (1944). Samples of the intergranular atmosphere were taken through lengths of steel capillary tube (inside diameter 0·9 mm.) inserted into the grain in a similar manner to the thermocouple ropes and subsequently withdrawn 1 m. at a time as the samples were taken. Samples were taken in evacuated 100 ml. bottles and the carbon dioxide concentration in these was estimated gasometrically.

* An improved form of this sampler has been described (Lucas & Glover, 1946).

The sampling point was chosen where the grain was warm to the hand 70 cm. from the middle of one side. Grain samples were taken at 1 m. intervals in depth down to 17 m., and also at the surface and at depths of $\frac{1}{2}$ and $1\frac{1}{2}$ m. In addition, a single sample was taken through the spout from the bottom of the bin.

Temperature measurements were made below the sampling point at 1 m. intervals in depth from $\frac{1}{2}$ to $11\frac{1}{2}$ m. and also at 25 cm. intervals from 10 to 410 cm. depth. A series of temperature measurements was made close to one side of this bin; at no depth did temperatures in this series exceed 18° C.

Samples of the intergranular atmosphere were taken at 1 m. intervals in depth from 1 to 14 m.

Data

All physical and biological data are presented graphically in Fig. 1. There is a discrepancy between the temperatures given by the short thermocouple rope (which gives temperatures at 25 cm. intervals) and the long rope (which gives temperatures at 1 m. intervals) although both were inserted at the same point. This probably indicates that one of the ropes was inserted at an angle to the vertical so that it was not quite coincident with the other.

The numbers of insects other than *Laemophloeus* found in all the samples was very small indeed. The records are given in Table 1; it will be seen that for all practical purposes the population of the grain was a pure *Laemophloeus* population.

Table 1. *Numbers of insects other than* Laemophloeus, *and also mites, found in the* 23 *samples taken from bin* 21. *Each sample weighs approximately* 200 *g.*

Insect	Found at first examination	Found during breeding out
Calandra oryzae	1	3
Calandra granaria	0	1
Rhizopertha dominica	0	2
Ptinus tectus	1	1
Psocids	0	21
Tribolium sp.	1	0
Mites (sp. undet.)	2	3

The species of *Laemophloeus* involved in this infestation appears to be *L. minutus* Oliv., but the female genitalia differ somewhat from those described by Reid (1942). In particular, the spermatheca of the present species is considerably more steeply curved than that figured by Reid for *L. minutus*.

Occurrence of insects within grains

Before this investigation it had been thought that larvae of *Laemophloeus* were normally free-living so that the total population could be determined by a single examination of the infested material. At the

first examination, however, a striking discrepancy became apparent between the rates of carbon dioxide production of some samples and the numbers of free-living larvae which were found in them. In no sample did the number of such larvae exceed 50 per kg. (and in most the number was considerably less) but the carbon dioxide figure indicated in some cases (notably the samples from 1 and 2 m. depth) a far greater population than was apparent, an observation which could only be explained on the assumption that a considerable population was intragranular and hence not directly observable.

Although only a very small proportion of the larval population was free-living at the first examination of the samples, much larger proportions appeared during the course of breeding out. The reason for this is not apparent but it is interesting to note that the proportion of the total population of each sample which became free-living during breeding out varied considerably in different samples. A smaller proportion became free-living during the breeding out process when the number of insects emerging from a particular sample was larger. There is evidence from studies of *Laemophloeus* in the laboratory

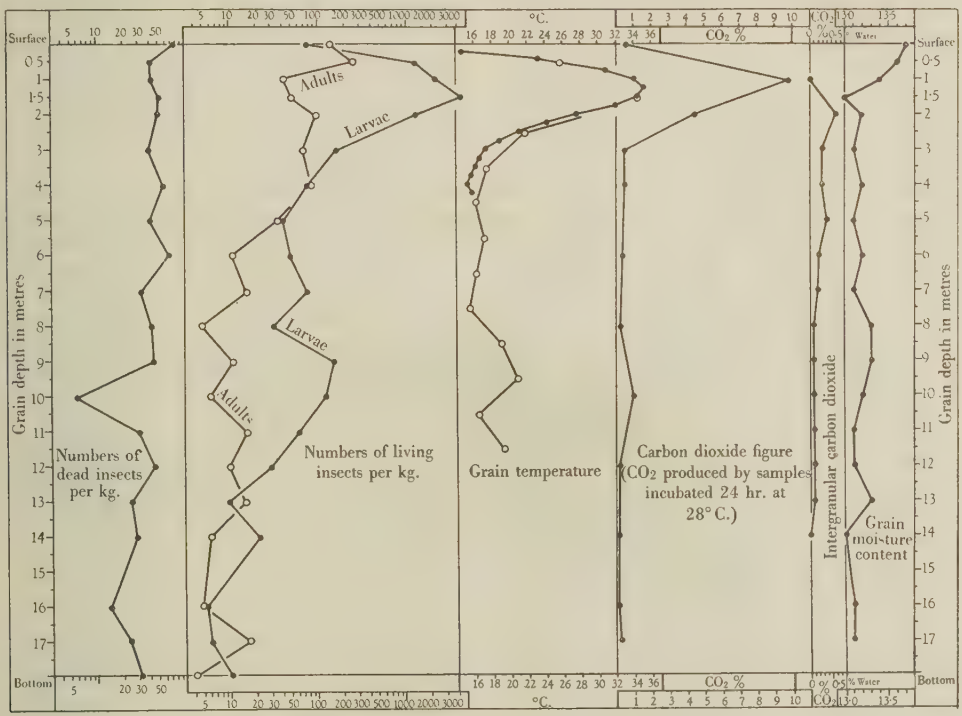


Fig. 1.

Breeding-out data (see Fig. 1) revealed that there was in fact a *Laemophloeus* population large enough to explain the high carbon dioxide figures. Hence the discrepancy is explained by the fact that the samples contained a large proportion of insects living within the grains. Dissection of some 200 grains showed that the infestation was limited to the germs of the grains and in these most larval stages and pupae were found. This evidence that *Laemophloeus* sp. may be largely intragranular in infestations of wheat has now been confirmed by observations at the Pest Infestation Laboratory that *L. minutus* may pass part or nearly the whole of its life history within wheat grains.

The distribution and source of the population

Fig. 1 shows that there is a close correspondence between the grain temperature and the distribution of the population. This is evident not only in the region of densest population, between the surface and 3 m. depth, but also in the deeper, subsidiary population between 8 and 11 m. depth. There is no

that the adults tend, under some conditions, to be cannibalistic and it may be that the reduced number of free-living larvae found in the more densely populated samples can be attributed to this habit.

doubt that the raised temperatures are a result of the high *Laemophloeus* populations and not a primary cause of them. Nevertheless, as Oxley & Howe (1944) point out, there is an interaction between insect populations in grain and the heat which they produce, since a rising temperature causes an increase in multiplication rate and metabolic rate, so that a self-accelerating process is initiated which rapidly leads to the development of lethal or at least repellent temperatures. The conditions in both centres of population are typical of stages in this process. Between 8 and 11 m. depth an early stage exists with the population increasing and producing a small rise in temperature. Between the surface and 3 m. depth a much later stage exists. The temperature exceeds 35° C. at one point and in this region the number of adult insects is considerably reduced in spite of the very large number of larvae. This strongly suggests that there had been a considerable migration of adults away from the hottest point to produce the two peaks at $\frac{1}{2}$ and $1\frac{1}{2}$ m.

The range of grain water content is small but it shows two peaks, one in the surface region and the other, much less definite, between 8 and 9 m. depth. Each peak is thus just above a zone of high population and temperature. Such a distribution of water is certainly produced by translocation under the influence of a temperature gradient and the direction has been chiefly upward as a result of local convection currents.

The concentrations of intergranular carbon dioxide are generally low and not clearly related to population. The fact that a negligible concentration was found at 1 m. depth in the hottest and most populous region could perhaps be explained by postulating an intense local convection in which replacement by cold air was from the sides of the bin rather than from below. No other evidence exists, however, for such a localized convection. The higher concentrations found below the hottest region may be due to a very slow general convection in the intergranular atmosphere between the lower and upper zones of high population.

The distribution of dead adult insects in the samples is interesting. Where the living population is high the number of dead is generally above average but in the deeper regions of the bin, where the living population is very low indeed, the number of dead remains considerable and shows no sign of being related to the density of the living population. This persistent residue of dead insects in the regions which, at the time of examination, supported only a very sparse population of living insects, must have arisen from a past population. This past population was probably not more dense than the present one and must have lived in the grain for a considerable time in order to have produced the observed residue of dead animals.

This throws some light on the probable source of the infestation. In the 8 months during which the grain had remained undisturbed in bin 21 it is unlikely that the observed deep, central, infestation could have developed from an initial infection originating at the walls of the bin or the surface of the grain. Either of the latter sources would probably be apparent in the distribution of the November population. It is more likely that the grain had long contained a very light infestation, fairly evenly distributed, which was the source both of the present dense infestation and of the dead insects in the sparsely populated areas.

If this hypothesis is correct, the observed distribution of the population, which was primarily concentrated between $\frac{1}{2}$ and 3 m. depth and secondarily between 8 and 11 m. depth, remains unexplained. It is known, however, that insect populations in bulk grain always tend to build up localized patches of high density as a result of the self-accelerating process of heat production referred to above. As Oxley & Howe (1944) point out, a result of this is that very small differences in the density of the original population, or in the favourability of the original environment, become greatly exaggerated. Similar small differences in the state of the infestation at the time when bin 21 was loaded may have been responsible for the final irregular distribution of the population.

Our thanks are due to Mr W. McA. Gracie, Director of Infestation Control, Ministry of Food, for the active collaboration afforded by his branch without which this investigation could not have taken place. In particular we wish to acknowledge the assistance rendered during the process of sampling the bins by the following officers of the Infestation Branch of the Ministry: Dr J. A. Freeman, Dr P. M. Butler and Dr E. W. Bentley. We also wish to thank Mr R. S. Glover of University College, Hull, who assisted in the laboratory work. This work forms part of the programme of research of the Pest Infestation Laboratory of the Department of Scientific and Industrial Research and this paper is published by permission of the Department.

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On making measurements in silo-stored grain

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(With Plate 11 and 4 Text-figures)

A sectional tubular steel sampler has been designed, with which samples of grain have regularly been obtained at 1 m. intervals to depths of 15 m. in normal grain. A new form of this sampler permits reclosing of the sampling ports after filling, so that contamination of the samples can be avoided. Associated records of the grain temperatures and samples of the intergranular gases have been obtained at similar depths. Certain precautions to be observed in sampling from silo bins are emphasized. Observations are reviewed which show the extensive effects of granular segregation during movement. Material, such as extraneous seeds, holed grains, etc., segregates so that (*a*) it is delayed in the outfall whilst emptying bins, (*b*) it is more concentrated in the central column of the bin after filling, (*c*) in direct sampling it is scarcer in the first part of the sample to fall into the container, with the implication that the sample as whole is deficient in such material. Larger objects settle at the surface of the grain on a moving belt, whilst smaller ones sink between the grains. The consistency of the results suggests strong reasons for a reconsideration of the principles of sampling, either direct or indirect. Meanwhile, samples obtained by any one method are likely to prove fairly consistent *inter se*.

Introduction

When the necessity arose in 1942 for making measurements at various depths in silo-stored wheat, there appeared to be no recognized methods other than those used by the trade for general examination of the superficial grain. The problem was approached, therefore, as a new one, although we were grateful for the opportunity to draw upon the experience gained by members of the Pest Infestation Laboratory, Department of Scientific and Industrial Research, Slough, during their investigations of wheat in floor-storage; we should particularly like to acknowledge the value of discussions with Mr T. A. Oxley of that laboratory.

Methods have been devised for obtaining grain samples, together with samples of intergranular gases and temperature observations. Whilst developing these methods, evidence was obtained regarding the extensive granular segregation which occurs in moving grain, and some account of these results may be of value to workers in this field. Meanwhile, an apparatus for collecting grain samples had been devised in the laboratories of the Board of Grain Commissioners for Canada (Anderson & Martin, 1943), to which reference will be made later.

Methods of sampling and measurement

General

Estimates were needed of conditions throughout the whole bulk of grain, with some details of local variation. Whilst it then seemed likely that the first requirement might be met by taking serial samples during the emptying of a bin, the second required direct sampling. It was primarily necessary to obtain representative grain samples in a vertical series as deeply as possible in the bin. The possibility of horizontal variation demanded that samples

be also taken in different vertical lines for comparative purposes. The varied methods of silo and bin construction presented certain problems to be solved in sampling, and consequently determined some of the principles which were adopted.

Silo bins are normally covered, and have only one manhole through which the grain may also be poured; this manhole (and the grain entrance) may be central or eccentric, and is usually so small that only one vertical series of samples can usefully be taken through it. There is normally no convenient means of approaching the grain surface in an incompletely filled bin, and the air in such a bin may be hot and foul. If the manhole is small it may be impossible to work on the grain surface, and at times this is undesirable since important evidence may be disturbed.

Preliminary surveys were made with the sampling spear designed at the Pest Infestation Laboratory for use in floor-storage (Oxley & Henderson, 1944). A larger head, collecting about 100 g., and additional extension rods were used; a depth of 15 m. has been reached in both Manitoba and Plate wheats. Such a sampler may also be used for taking superficial samples from incompletely filled bins, although it cannot be inserted far into the grain under such conditions owing to the undue strain on the unsupported extension rods. For most purposes it had the disadvantage of taking only one sample at each insertion, and even approximately vertical sampling at depths greater than 5 m. could not be guaranteed.

The early deep-sampler

A more robust sampler was designed to take a series of vertical samples, at 1 m. intervals, during each insertion. It comprised a solid spearhead and a series of steel sampling tubes joined by small brass couplings, each with a pair of sampling ports. Within the brass couplings slid a series of brass plungers, connected by brass rods, which could close the sampling ports when at rest. The units were coupled together and progressively inserted until the maximum depth was reached, when the plunger system could be raised, thus opening the ports and permitting the samples to be taken. The samples were then poured into bags one by one as the units of the sampler were extracted, and subsamples could be taken immediately and placed in airtight tubes for subsequent water-content analysis.

The sampler could usually be pushed by two operators to a depth of 8–10 m., after which mechanical assistance was required. Whilst a suitable lever system might have been designed, a convenient ready-made aid was found in a pair of 2–3 sheave pulley blocks and 60 ft. of rope. The upper 3-sheave block was modified to sit on the upper end of each unit as it was added, whilst the lower block was hooked to a bar beneath the bin roof. Steady hauling on the rope then moved the sampler deeper into the grain.

Hundreds of samples, each about 200 g., have thus been taken in Plate and Manitoba wheats under various conditions of infestation, between the surface and 15 m. depth; the maximum depth reached was 18 m. in Manitoba wheat of about 13% water content. Whilst it is not possible to guarantee an absolutely vertical series of samples, all the evidence suggests that a deviation of less than 1 m. in 15 may be expected when working in the centre of the bin; there is possibly a greater tendency to 'wander' when working near the side of the bin owing to the proximity of the bin wall. According to the position of the manhole, samples have been taken in the centre or near the sides of the bin, and also along diagonal or oblique lines until the spear reached a wall. Owing to its relative rigidity, this gear may be used for sampling in incompletely filled bins, although penetration must be limited to lesser depths to prevent straining the unsupported length of metal tube. It has normally been used to reach depths of 15 m. by two people, although the help of a third was invaluable at times. We have not kept regular time records of the different procedures, but on one occasion, with three workers, grain samples (with sub-samples for water content) were collected from two vertical series to 14 m., and one diagonal set meeting the wall at 15 m., and stored away in 100 min.

There is no doubt that the greatest fault lay in the impossibility of reclosing the ports after sampling. If there should be a dense surface population, it may be difficult to eliminate the possibility that some of the insects found in the deeper samples may be surface contaminants. Examination of the evidence has always suggested that such contamination must have been slight, but, when the opportunity arose to construct another sampler, we felt it essential to overcome this difficulty and to make some other improvements. In particular, there was need for a plunger system which could be assembled quickly in poorly illuminated conditions. It was also thought that penetration of the grain would be easier if the bulges formed by the external brass couplings were eliminated by screwing the sampling tubes together directly. Before constructing a new sampler, information arrived regarding the design of the sampler made in Canada (Anderson & Martin, 1943), and it is useful to consider its main features, since we chose again the general principles of the design of our old sampler in preference to the ones adopted by them.

The Canadian sampler

Anderson and Martin employed a system of screw insertion, in which a series of tubular sampling units

was connected to a terminal 'augur' section corresponding to the spearhead in our gear. The principle of screw propulsion had been considered when designing the early sampler. Whilst it had the great advantage of providing the sampling and propulsion gear within one system, it was rejected since: (1) the rotation of a comparatively large screw in the grain surrounding the sampler, followed by a variable period before opening the sampling ports, might induce some avoiding, or even attractive, reaction on the part of the insects; (2) it was anticipated that the screw method would be slow and inconvenient during the 'easy' stages of insertion; and (3) the strains set up within the gear were likely to be more serious than in a gear depending upon vertical pressure for insertion. Otherwise screw propulsion seemed the obvious method to adopt, and the description of its working shows how it may have succeeded in places where ours would have failed: particularly in situations commoner in Canada than here, where great bulks of deep grain are stored and there is little room for overhead gear. A greater depth has been reached with it, although in general its normal working depths were less than ours, and a larger team of workers has been considered desirable and normally used. The Canadian workers appear to confirm some of the objections which we anticipated and, in view of the first objection raised (i.e. disturbance of the surrounding grain), we decided to try to develop further the method which we had first adopted. It should be noted that the Canadian apparatus, like ours, had the objection that no means was provided for reclosing the sampling ports. There is no mention in their report of any attempt to obtain records of any environmental factors other than water content.

The new deep sampler

Essential details of the new sampler are shown in Text-fig. 1. All parts are made of steel. It includes (*a*) a spearhead, (*b*) a series of unit sampling sections, (*c*) handles for insertion and extraction, and (*d*) a small handle for operating a plunger system.

The spearhead is tapered and fitted with female threads (surrounding a 'stop' on which the plunger system may rest) for attachment to the series of sampling units.

Each sampling unit comprises:

(1) a solid drawn tube (*A* in Text-fig. 1) with male threads at its lower end and female at its upper end, and having paired sampling ports (*B*), with bevelled edges, situated below the upper threads; and

(2) a rod (*C*) connected at its upper end to a plunger 'skirt' (*D*), with two projecting clips (*E*), and at its lower end to a plunger 'head' (*F*), with a circular groove (*G*).

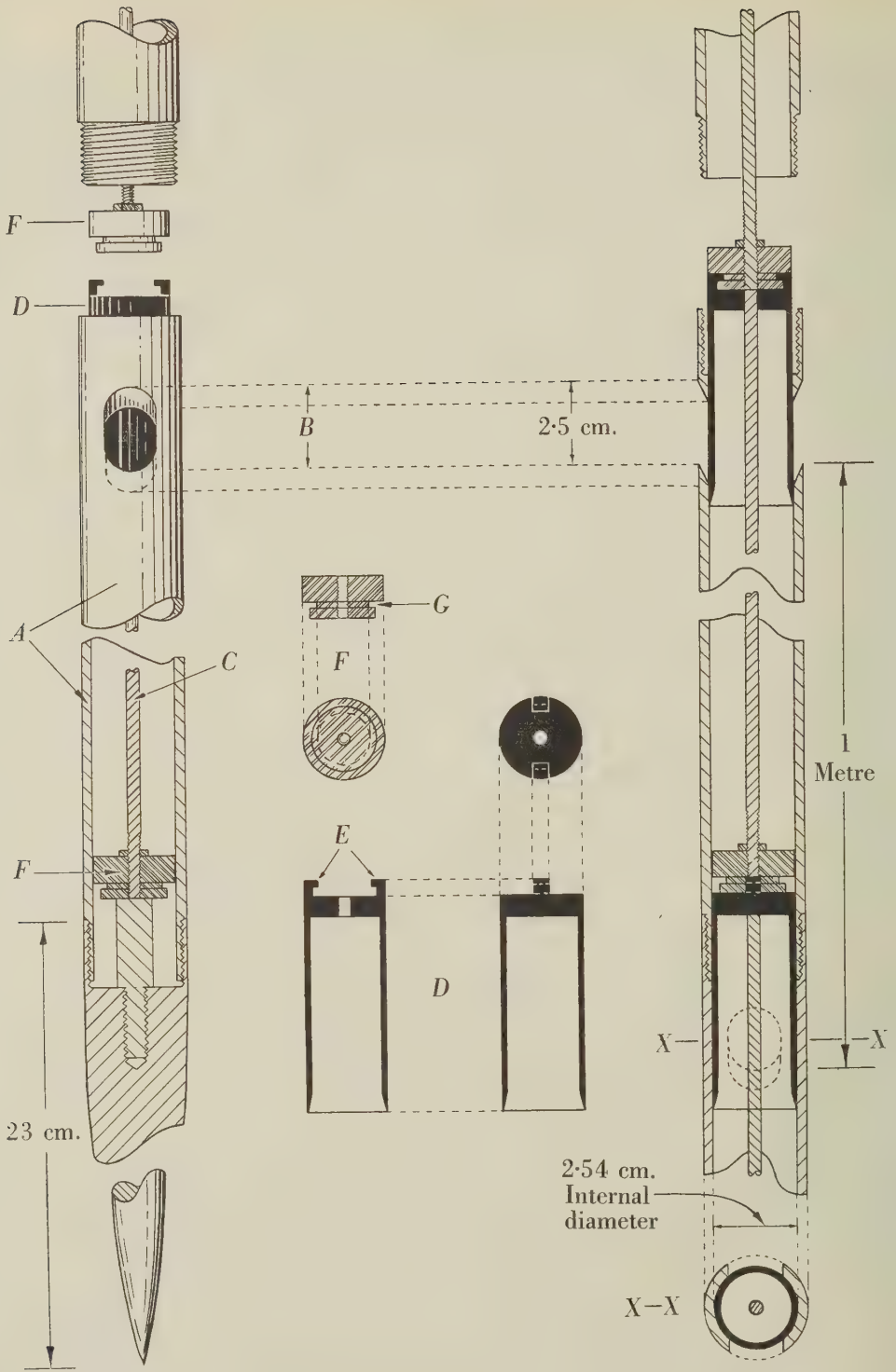
The plunger 'skirts' and 'heads' are machined to slide-fit within the steel tubes so as to cover and uncover the ports. The clips on the 'skirts' are machined to slide within the grooves on the 'heads'; when so joined a 'skirt' and a 'head' form a complete plunger. All parts are interchangeable.

In assembly, the male threads of a sampling tube are screwed into the female threads of the spearhead, so exposing the clips of the appropriate plunger 'skirt' at the upper end of the tube. This 'skirt' forms the lower part of a complete plunger when the plunger 'head' projecting from the lower end of the tube is slipped between its clips (Pl. 11, fig. 1*a*). This tube may then be screwed by its male threads into the upper end of the first one. The assembled unit is then pushed into the grain and the process repeated. The sampler is so constructed that the sampling ports are 1 m. apart when the gear is assembled, the bottom sample being collected 1 m. from the spear head. When the plunger system rests on the stop in the spear head, as in descent, the sampling ports are closed. When required, the plunger system may be lifted (by a small handle, fitting into the clips of the top plunger 'skirt') until the top port is well open, when the grain will pour in (Pl. 11, fig. 1*b*). The ports are closed by firmly pushing the system back again, any obtrusive grains being cut by the sharpened edges of the plunger 'skirts'.

As with the earlier sampler, this one may be pushed by hand at first, using a large handle which slips *over* the top sampling tube without damaging the exposed clips. A pulley block or lever system may be used as a mechanical aid. Another handle, screwing *into* the top tube, is used for extraction (Pl. 11, fig. 1*c*). As each tube is removed, the grain is retained by the plunger parts at each end, so that no loss occurs; it may be poured into bags when desired (Pl. 11, fig. 1*d*).

Simple observations on a unit sampler in a laboratory model bin show that, with both holes opening together, the grain is drawn from a region extending for some 10–12 cm. above the bottom of the port. Pl. 11, fig. 2 shows photographs of the deformation produced in a layer of stained grain after opening the ports. If only one of the ports should be open, a sample is drawn from a greater vertical distance. Probably some 15 cm. is the maximum height from which grain could be drawn into the sampler even if one hole were accidently blocked in the bin. In view of the angle of repose in grain, it is expected that rather similar horizontal distances will apply.

The new sampler has just been completed and only one test has been possible (in Manitoba wheat of water content 13·5%). It was possible to push it down to only 14 m., probably owing to the lack of a sufficiently fine polish on its surface. We found,



Text-fig. 1. The new deep sampler (pp. 295–297). On the left is the spearhead and bottom unit sampler assembly; on the right a standard unit sampler assembly, in section. The plunger parts are shown in section in the centre.

however, that the early insertions of the old sampler progressively smoothed the surface, and greater depths should be reached when the new one is burnished. The samples were of the order of 350–360 g. This test was important in showing that the plungers completely closed the sampling ports after filling. On the other hand, experimental sampling through an artificial surface of stained wheat-meal and whole wheat grains produced one stained whole grain in each of two samples out of fourteen taken beneath the surface, and traces of the meal in every one. There is no doubt that there was an unexpected source of contamination almost certainly due to the collection, as the sampler descended, of a small amount of surface material within the grooves between the lower bevels on the sampling ports and the closed plungers. Such traces might be carried down with the sampler and would then fall into the tube when the plunger was raised. Such slight contamination must have occurred in the old sampler, and there should be several ways of preventing it in the future. The bevels were merely introduced to ensure that, if the sampling ports could not be closed, complete filling of the sampler should occur as soon as it began to be extracted. Since the ports can be closed, the lower bevels could be reversed and this risk of contamination avoided.

Temperature recording and gas sampling apparatus

Records of temperature and samples of the intergranular gases were required to provide data regarding the general conditions of storage, and of the environment of any infestation which might be present.

The first was made possible with the collaboration of the Biological Section of the Pest Infestation Laboratory, which supplied for our use thermocouple ropes built by Mr J. D. Jones to the design of Oxley & Henderson (1944), but 100 ft. long. Two types were used: (*a*) with six couples at 5 m. intervals, and (*b*) with nine couples at 2 m. intervals; the latter was undoubtedly the more convenient arrangement. In each the wires ran to a multiple plug for insertion into the special potentiometer designed by them (p. 3 of their account), and kindly loaned for our work. We normally required records at 1 m. intervals, and obtained them by first inserting the ropes to the required depth, leaving them for at least 20 min. so that they might reach temperature equilibrium, then making the record and raising the whole rope through 1 m. so that another reading could be made. In view of the great strains set up in reaching 15 m. depth, these delicate ropes have worked extremely well. 2 m. ropes such as Oxley & Henderson have described were also used, their couples spaced at 25 cm. intervals providing valuable evidence of temperatures near the grain boundaries.

Samples of the intergranular gases were collected through stainless steel capillary tubing into evacuated glass bulbs, after the residual gases in the tubing had been extracted by means of a gas syringe. This steel tubing had been specially designed for the Pest Infestation Laboratory, and worked extremely satisfactorily. The carbon-dioxide content of the gases was estimated by the methods of Oxley (1944).

We normally inserted the thermocouple rope and capillary tube together by means of the extension rods first used for inserting the Oxley sampler (p. 2). Both were attached to a special solid spearhead for this purpose, the rods being withdrawn immediately after insertion and the thermocouple rope left in position to reach equilibrium. The samples and temperature records were thus in associated pairs, although it could not be guaranteed that they were from just those spots in which grain samples had been taken with the deep sampler. The probable deviation is not likely to matter greatly except when working close to the bin walls, where temperature changes may be very sharp. Diagonal and oblique, as well as vertical, data may be obtained.

Whilst the thermocouple ropes and the capillary tubing have also been inserted with the grain sampler, after attachment to its spearhead, this method led to undue strain on the thermocouple ropes (presumably owing to grain pressures), and demanded a longer period to reach temperature equilibrium owing to the bulk of metal in this sampler.

Survey procedure

Experience gained regarding conditions in covered (but by no means airtight) bins suggests that the following precautions are necessary if the most informative results are to be attained. Before removing the manhole-lid, samples of the superficial and subsurface gases may be obtained with the minimal losses, either through the small holes commonly found in the lid, or by very slightly opening it. Samples in the superficial grain should be collected immediately after opening the lid, and temperatures in the subsurface grain should be recorded by mercury thermometers, in order to minimize the changes which may quickly occur after opening a heating bin. The routine processes may then proceed, although it may be better to take first the temperature records and gas samples at required depths before the longer process of taking grain samples.

Time will be saved if sterilized sample bags or tins are arranged in numerical order, and all electrical equipment checked before the survey. Gas bulbs may be evacuated with a 'Hyvac' pump in the laboratory, or with a portable pump at the silo. Tapping the sampling tubes will remove any large

insects which lodge there when withdrawing the samples, but smaller ones may become lodged and it may be desirable that the tubes be cleaned with a 'pull-through' between insertions.

Detailed results from infested bins have been filed with the Pest Infestation Laboratory, Slough, Bucks; it is hoped that a report will be published later.

Segregation in moving grain

Sampling whilst the bin is emptied

It had been expected that the taking of serial samples during the emptying of a bin would at once provide estimates of overall conditions within the bin, as well as checks on the validity of different methods of direct sampling. The application of this method to an infested bin of Plate wheat gave results which were surprising and probably important.

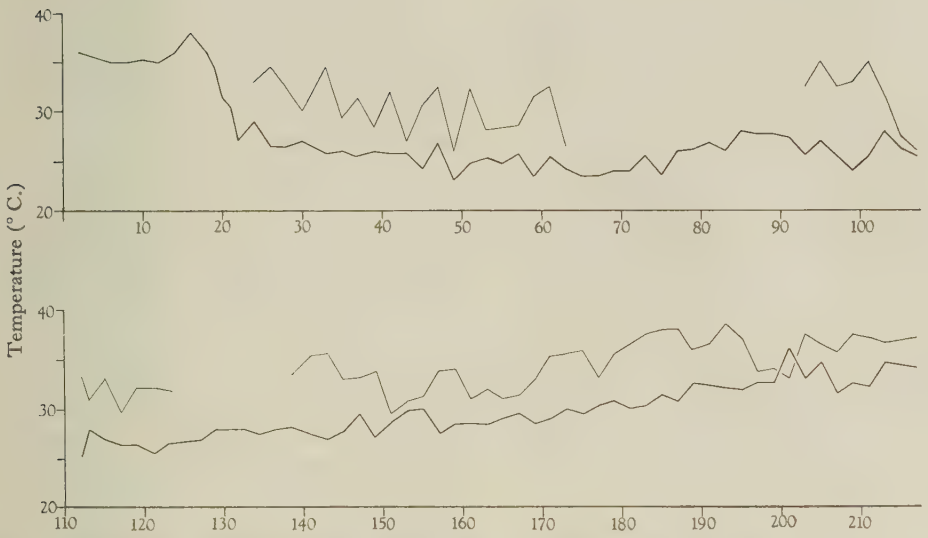
This bin was at first partly full of infested wheat but, a day before emptying, it was completely filled from a much less infested stock at a lower temperature. Direct samples of grain were collected in the central column of the upper three-fifths of the whole bulk, but no deep records of temperature were obtained on this occasion.

The bin was emptied through a sloping metal pipe into a worm conveyor. A trap in a vertical section of the pipe, just above the worm, provided a convenient sampling point, and arrangements were made to record temperature variations there and to take successive 20 g. samples from the centre of the falling stream. After a preliminary stage of high temperatures in which similar records were obtained from the centre and edges of the stream, the temperature in the centre suddenly became relatively cooler than at the edge; records are shown in Text-fig. 2. This difference persisted, with one exception, until the bin was practically empty (the last portion being unexamined); a maximum difference of 10° C. was obtained in the course of seventy-three paired observations. It was obvious that samples taken in the cooler central grain might be different from those at the edges, and twelve paired samples taken in the two places later showed the grain near the edges to have contained larger numbers of several characters (extraneous seeds, insects and holed grains) than their central partners. A more detailed series of fifty-four pairs of samples was taken alternately from the centre of the pipe and from the worm just after the grain had fallen (and presumably mixed) in it. This showed the central pipe samples to be irregularly deficient in all the characters examined, except a yellow wheat contaminant and very small insects (*Laemophloeus* spp. and psocids). The mean results are shown in Table 1. Among the extraneous seeds, the difference was slight in the oats, greater in buckwheats, and greatest in the crucifers. Representative sets of data are given in Text-fig. 3 for crucifers and for the immature stages of *Rhizopertha dominica* which ultimately bred out of the grain. Table 1 shows further that, for these and other forms, the differences were greater on the whole when the temperature differences were the greater.

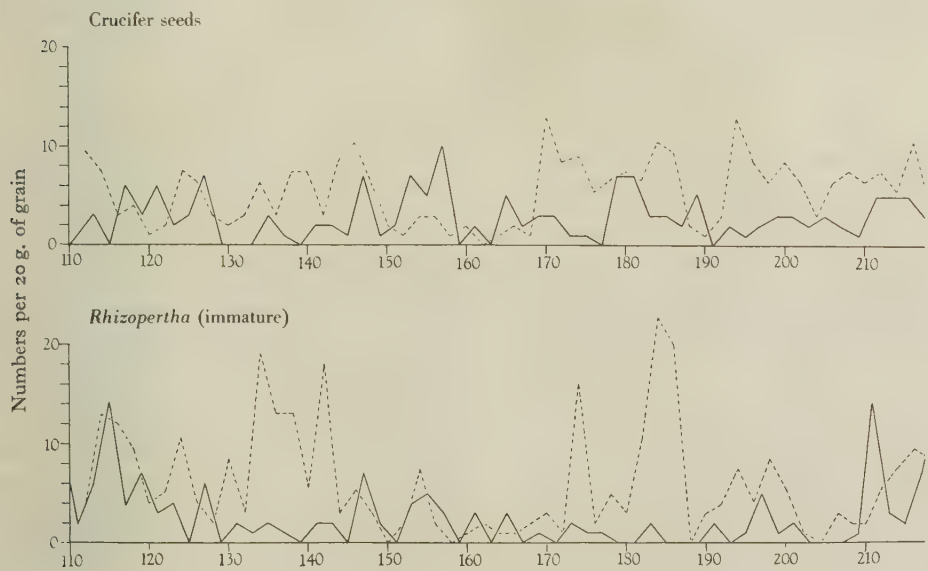
The existence of such a difference is itself important. It has not been possible to examine another bin of wheat in a similar manner for confirmation, but these results alone show that the most careful

Table 1. *Giving the total quantities of various characters found in the fifty-four central pipe samples, in comparison with those found in the fifty-four corresponding worm samples, together with the appropriate worm/pipe ratios for equal weights of grain. Alongside are shown the corresponding ratios for those parts of the outfall where the temperature differences in the pipe were less than* 5° C., *and those where it was more than* 5° C.

	Total nos.			W/P when temp. diff.	
Character	Worm samples	Pipe samples	Worm/Pipe	<5° C.	>5° C.
Weights (g.)	1036	1087	—	—	—
Oats	149	134	1·2	1·1	1·3
Buckwheats	128	86	1·6	1·0	2·0
Crucifer seeds	286	161	1·9	1·2	2·3
Yellow grains	77	86	0·9	0·7	0·9
Holed grains	2217	1733	1·3	1·1	1·5
Calandra adults	6	2	3·1	2·0	(4/0)
Calandra immature	12	3	4·2	3·0	(8/0)
Calandra dead	115	49	2·5	1·8	2·2
Rhizopertha adults	13	11	1·2	0·6	2·4
Rhizopertha immature	311	128	2·5	1·7	2·7
Rhizopertha dead	175	99	1·9	1·3	2·2
Other adults	20	19	1·1	0·5	1·6
Other immature	4	12	0·4	0·5	0·4
Other dead	9	14	0·7	0·2	1·2



Text-fig. 2. Showing a series of temperature records taken in the centre (thick line) and at the edge (thin line) of a stream of grain falling through the outlet pipe of an emptying bin. The serial numbers denote the successive readings at 3 min. intervals. Results from related grain samples are shown in Text-fig. 3.



Text-fig. 3. Showing the numbers of crucifer seeds (above) and immature *Rhizopertha* (below) collected from serial samples in the centre of the outfall pipe (continuous line) and in the worm (broken line). The serial numbers denote the successive samples and correspond with those in Text-fig. 2.

consideration must be given to the significance of any samples collected from a falling stream of grain, and that only samples from grain in which remixing has occurred may give an adequate estimate of conditions within the whole bulk. Detailed consideration cannot be given here to the manner in which the differences arose, but the data suggest that: (*a*) in this type of bin the upper grain 'funnelled' out of the bin before the lower, (*b*) the products of this 'inversion' were most evident in the centre of the falling stream, whilst irregular infiltration of lower (and, in this bin, warmer) grain contributed to the edges of the stream, whilst (*c*) in general objects such as seeds, insects and holed grains (some of which are known to be less dense than wheat, and most of which may be so) segregated in the outfall relative to the denser whole grain, so that they tended to be diverted to the edges of the stream and their proportions in the later outfall were variably excessive. This has the corollary that in 'turning' grain an undue amount of such material is likely to be placed at the top of the second bin.

Sampling whilst the bin is filled

Another type of segregation was met when comparing small samples from grain moving on a belt conveyor just before entering a bin, with direct samples taken immediately afterwards in the central column of the bulk. Thirteen comparable pairs of samples gave numbers as follows:

	Adult insects	Infested grains	Dead insects	Oats	Buckwheats	Crucifer seeds
Belt	6	**65**	46	**50**	39	50
Bin	6	39	**161**	29	**85**	**107**

Excluding adults, only four out of 65 pairs of samples were in disagreement with the relationship given by the means in this table, so that the belt samples were giving consistently high numbers for certain objects, and low ones for others. Since the belt samples were necessarily taken from the surface it appeared that the oats and the grains containing immature insects were unduly abundant there, whilst the other characters, except adults, were too few.

In order to test this, an experiment was made in which similar samples taken from the surface grain on a belt were compared with larger samples, taken by scooping more deeply into the grain, which might be expected to collect more of the deeper grain. For a number of pairs of such samples (between eight and twenty-four, according to the character examined), the following means were obtained:

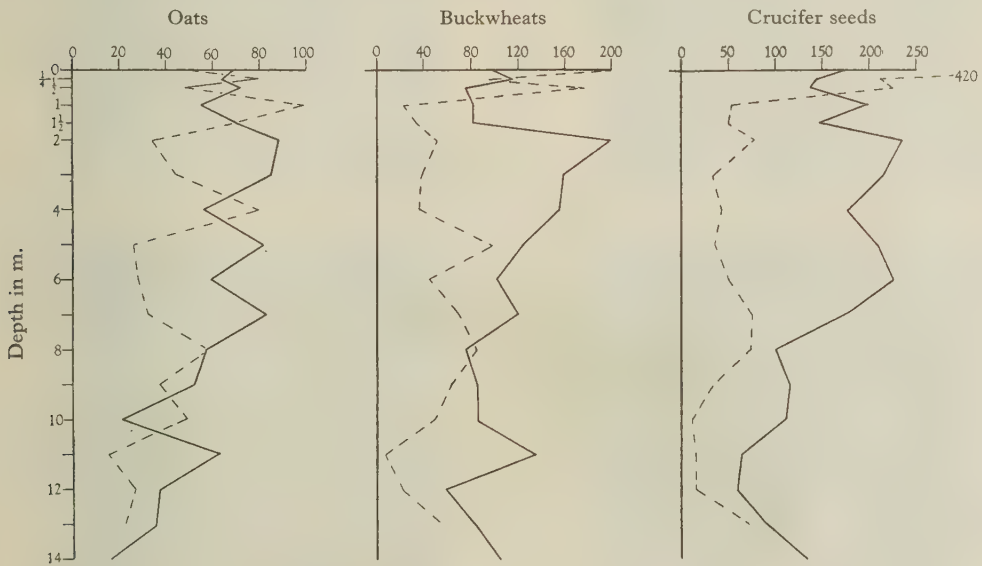
	Adult insects	Infested grains	Dead insects	Oats	Buckwheats	Crucifer seeds	Holed grains
Surface grain	36	132	90	**43**	33	25	**5·7%**
Surface and deeper grain	35	138	**127**	28	**63**	**69**	4·0%

There were no significant differences between the numbers of adult insects in the two methods, nor of infested grains. Of the other characters, however, the oats again proved to be more and the buckwheats, crucifers and dead insects less abundant in the surface layers; the holed grains were distributed in a manner similar to the oats. It appears, therefore, that segregation occurs on the belt.

Before discussing the explanation of this distribution, another type of segregation following on the filling process should be mentioned. Preliminary examinations with the Oxley sampler had suggested that a number of characters might be more abundant in the centre of the bin than at the side immediately after filling, and the opportunity was taken in a bin with two apertures to test this more thoroughly. The numbers of extraneous seeds are shown in Text-fig. 4, and prove conclusively that in this bin, also, the numbers were much higher in most of the central grain; near the surface, however, this was reversed, as had also appeared earlier, although it was not then thought to be significant. It was not possible to take samples early enough in this bin to observe whether a similar distribution applied to the living insects immediately after filling, but the dead insects and the holed grains were similarly distributed; the preliminary results with the smaller sampler suggested that this might apply to living insects. It should be noted that the difference was much the least in the oats, more evident in buckwheats and most evident in crucifers. The highest numbers of extraneous seeds, etc., were found near the surface of the grain (particularly near the side). This has tended to be the case in most bins where there have not been sharp irregularities in the distribution, such as might represent extreme heterogeneity in the bulk from which they were derived.

The suggested explanation concerns the densities and sizes of the materials relative to the whole grain. In falling distances of 50–100 ft., the denser materials may be expected to fall faster and to roll farther down the coned surface of the bulk, i.e. the less dense ones would lag and be more abundant in the centre. This will be reinforced by any tendency for smaller materials (crucifers, dead insects, etc.) to lodge in the intergranular spaces after impact. It is significant that of the three bins in which segregation of this type was found, all had a central entrance for

the grain; the only one providing data which does not show this segregation had its grain entrance approximately midway between the centre and the corner of the bin, so that the distribution in the corner and centre might be expected to be fairly similar. The reversal within the top metre of grain is probably connected with the fact that in any column there are usually larger numbers of extraneous seeds, etc. near the surface of the grain. A possible explanation may be found in the probable production of clouds of the lighter characters near the moving surface of the grain during filling. If this should be extensive, then the inevitable settling of this material as the bulk reaches the top of the bin might well



Text-fig. 4. Showing the relative numbers per kg. recorded in the central column (continuous line) and in the corner (broken line) of a bin of grain.

result in larger numbers being incorporated at the edges of the cone, and would certainly result in larger numbers being found in the surface layers whether the bin were completely filled or not.

Whatever the explanation, the evidence for segregation during filling is conclusive, and demonstrates that even in a recently filled bin samples taken beneath one point are likely to give biased estimates of the conditions over the bulk; at later stages the possible biological developments provide further reasons for taking more than one set of samples. The results show further that the central samples with which we compared the surface belt samples (p. 300) were themselves biased, so that the comparatively smaller numbers of buckwheats, crucifers and dead insects found on the belt would arise partly in this way. Yet the excesses of centre over side were much less than the excess of bin over belt for these characters, whilst the oats were more numerous on the belt than in the bin samples. In view of the results found in the second belt test, it appears that the explanation lies in segregation through vibration during the movement of the belt, in which relatively large, but sufficiently light, objects such as oats and holed grains 'float' to the surface of the grain, whilst small ones like crucifer seeds sink in the intergranular spaces. It is uncertain how far infested grains are affected by these processes; adult insects do not appear to be affected, perhaps because they may cling to the grains. It seems likely that reconsideration and further investigation of the principles of sampling from conveyor belts is required.

Direct sampling

In view of these effects of segregation on grain in motion, it was thought that it might also influence the samples taken directly in the bin (or in any bulk of grain), in so far as the grain is set in motion by the sampling process. In order to test this, arrangements were made to divide each of the samples in one series from the early deep sampler into approximately upper and lower halves. This could only be done by pouring, in which further segregation might occur, so the successive samples were poured alternately from the lower and upper ends of the

sampling units. Thus, in one set, grain was sampled through the upper end and poured out of it, the effects of segregation tending to be neutralized; in the other, grain was sampled through the upper end, but poured out of the lower, the possible effects of segregation being reinforced. In general, the upper halves, as separated, contained excessive numbers of those characters which elsewhere had shown segregation. In detail, the differences between upper and lower halves were slight and irregular when the grain was poured out of the sampling end, whereas when it was poured out of the other end excessive numbers were found in the upper halves for all the characters except small insects. The respective ratios when poured from the upper sampling end were:

	Adult insects	Infested grains	Dead insects	Oats	Buckwheats	Crucifer seeds	Holed grains
Upper/lower	0·97	0·85	1·04	1·13	1·35	0·76	1·04
When poured from the lower end they were:							
Upper/lower	1·81	1·24	1·87	1·13	1·06	1·66	1·08

Thus, segregation did take place during the sampling process, and was reinforced, as suggested, by the second pouring when this was from the lower end. The first part of the sample to enter the tube appears to be deficient in certain characters, just as the first part of the grain to fall out of a bin appears to be deficient in similar ones, so that the principles of direct sampling need reconsideration. Unfortunately we were unable, before ceasing this work, to obtain estimates of the deficiency involved in this type of sampling; the ratios of numbers found in the upper and lower parts of the sample do not provide this, and the observations are recorded as a basis for future work. The data suggests that results from any one type of sampler should be comparable *inter se*, but that data from different types may differ appreciably. Probably few will be really representative, but segregation might be appreciably lessened in samplers in which the aperture is large in proportion to the volume, since the grain then tends to be projected into the container.

It is probable that both relative density and relative size are significant factors in these segregation processes. The general influence of segregation by density has long been known to the trade; Bealing (1939, p. 17) says 'the grains separate themselves according to their density, the heaviest are discharged first'. The present results show that the effects of segregation may be very extensive and, in the case of the outfall from an emptying bin, might possibly be applied to the separation of more from less infested grain.

Finally, it may be added that segregation must play a part in any subsampling processes, being a major factor in the difficulties which we, and probably other workers, have found in obtaining reliable subsamples during the estimation, for example, of the proportions of holed grains in large samples.

The work described above was carried out for the Department of Scientific and Industrial Research, as part of the programme of the Pest Infestation Research Committee, and this paper is published by permission of the Department.

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EXPLANATION OF PLATE 11

Fig. 1. Photographs illustrating the use of the new deep sampler: (*a*) introduction of lower end of one unit to upper end of preceding unit; (*b*) lifting the plunger system by its small handle, thus opening all the sampling ports; (*c*) large handle being used for extracting the whole sampler; and (*d*) depressing the plunger skirt to pour the sample into a bag.

Fig. 2. Photographs illustrating a horizontal layer of stained grains before (left) and after (right) opening the ports of a sampling unit. The white line is 1 in. long and is situated at the top of and between the two sampling ports, which were about 1 in. behind the visible grains.

(*Received* 12 *January* 1946)



Fig. 1*a*



Fig. 1*b*



Fig. 1*c*



Fig. 1*d*



Fig. 2 (left)

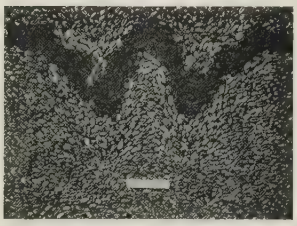


Fig. 2 (right)

LUCAS AND GLOVER—ON MAKING MEASUREMENTS IN SILO-STORED GRAIN

The design and performance of an atomizing nozzle for use with a spraying tower for testing liquid insecticides

By P. S. Hewlett, *Pest Infestation Laboratory, Slough, Bucks*

(With 2 Text-figures)

A nozzle, P.I.L. No. AN2, has been designed for, and used successfully in, the biological assay of insecticides in a heavy oil solution. This new nozzle is easier to adjust and gave a standard deviation of deposit one-seventh of that given by the nozzle described by Potter (1941).

Introduction

A spraying tower for the even deposition of controlled amounts of liquids on surfaces, insects, etc., has been described by Potter (1941). An important feature of this apparatus is the atomizing nozzle, the inner and outer cones of which can be centred relative to one another. It was found, however, that nozzles of Potter's design did not give sufficiently good replication of deposit for spraying insects during the biological assay of pyrethrum in a heavy mineral oil, e.g. Shell Oil P31. Another nozzle, P.I.L. No. AN1, was therefore designed, and this proved superior. Further improvements were thought possible, however, and these were embodied in atomizing nozzle P.I.L. No. AN2, the performance of which has been found highly satisfactory.

With the nozzle of Potter's design, it is very difficult to repeat a nozzle setting, i.e. on reassembly after dismantling it is very difficult to reset the inner cone at the same distance behind the outer cone; the cone-setting and cone-centring adjustments are not independent; and although the tips of the cones may be centred, they may not be longitudinally coaxial. It was thought that elimination of these disadvantages, without radical alteration of the diameters of the cones, might improve the spraying performance. Accordingly, AN2 incorporates the following features: (1) the cones are centred accurately to 0·0002 in. by means of screws acting on the outer cone, (2) the vertical position of the inner cone (nozzle setting) is positively controlled by a screw device, and (3) these two adjustments are independent.

Description of atomizing nozzle P.I.L. No. AN2

Figs. 1 and 2 are respectively a schematic longitudinal section and a plan of AN2. In neither figure have the diameters of the cones been drawn exactly to scale. In Fig. 1 the air injection tube, one of the centring screws, and one of the cap-retaining screws are shown, but in fact these parts do not lie in one plane, and their true positions are indicated in Fig. 2.

The nozzle is made of brass, and its main parts are the body, liquid feed stem, cap, and liquid suction tube. The lower ends of the liquid feed stem and cap constitute the inner and outer cones respectively. The upper part of the liquid feed stem bears an external 2 B.A. thread and the position of the liquid feed stem can be altered by turning the stem-adjusting nut, which is fixed into a boss on the body by means of two straight, steel nut-locating pins (with their long axes at right angles to the plane of Fig. 1), fitting tangentially into a groove on the outer surface of the stem-adjusting nut.

The lock nut and fibre washer are to prevent accidental alteration of the nozzle setting. The cap is tapered to a narrow apex to reduce possible eddying in the injected air stream. It fits loosely into a recess in the body, and its lateral position can be altered by means of the three centring screws. The thread on these screws is 5 B.A., and for fine adjustments they are turned by means of 2 in. tommy bars inserted into holes in the screw heads. The cap-retaining screws pass through holes which are large enough to allow some lateral movement of the cap when they are loosened. The end of the liquid suction tube dipping into the liquid reservoir is flattened and notched.

The dimensions of the cones are the same as those advocated by Potter (1941), except that the internal diameter of the outer cone has been increased, so as to reduce the accuracy with which the cones need to be centred. The dimensions of the cones, with tolerances, are as follows:

Inner cone, internal diam.: 0·0330 ± 0·0005 in.
Inner cone, external diam.: 0·0470 ± 0·0005 in.
Outer cone, internal diam.: 0·0675 ± 0·0005 in.

Adjustment of the nozzle

To centre the cones, the cap-retaining screws are loosened, and the tips of the cones brought flush with one another by rotation of the stem-adjusting

nut. The centring screws are turned until the cones appear to the unaided eye to be centred. The error in centring is then determined by means of a monocular microscope containing an eyepiece micrometer, and fine adjustments are carried out by turning the centring screws with a tommy bar until the cones are centred to the nearest 0·0002 in. If the cones are 0·0002 in. out of centre, the maximum and minimum widths of the annulus between the cones differ by about 4%. When the cones have been

Performance of the nozzle

The performance of the nozzle was investigated by means of distribution and replication tests, for which a gap of $\frac{15}{32}$ in. between the flange of the tube of the spraying tower and the spray table proved suitable (see Potter, 1941). Each distribution test consisted of weighing the deposits of Shell Oil P31 on $\frac{7}{8}$ in. square cover-slips disposed in the form of a cross over a filter paper 9 cm. in diameter. At spraying

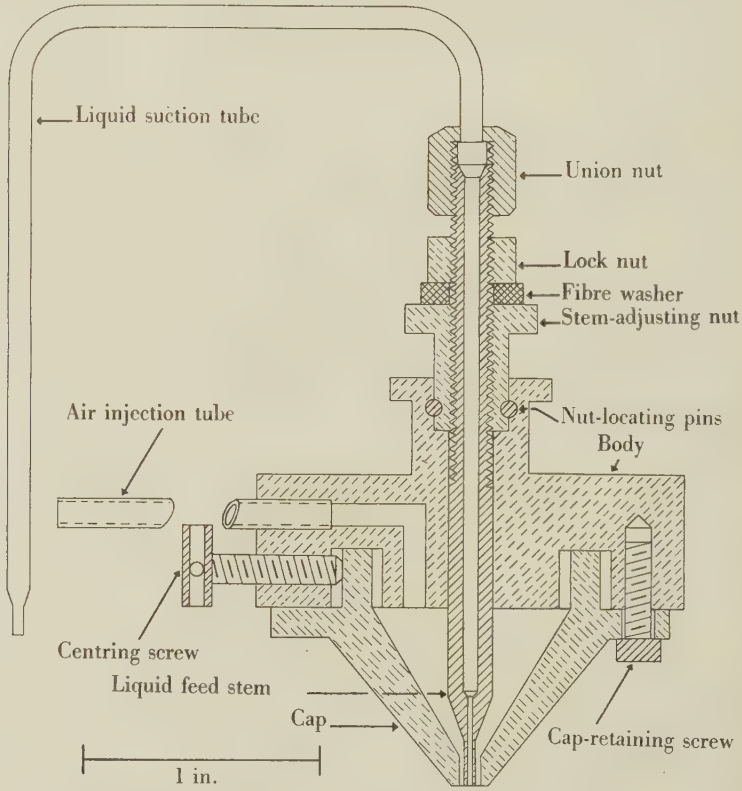


Fig. 1. Schematic longitudinal section of atomizing nozzle P.I.L. No. AN2.

centred, the liquid feed stem should not be rotated relative to the body, for, if the tip (inner cone) and shaft of the liquid feed stem are slightly eccentric, the centring will be destroyed.

Nozzle setting, i.e. the distance of the inner cone above the outer cone, is expressed as the number of turns of the stem-adjusting nut in moving the inner cone upward from the position in which the tips of the cones are flush. Each turn of the stem-adjusting nut moves the inner cone 0·0319 in.

pressures of 15 or 20 cm. of mercury, the distribution was highly satisfactory, with peripheral differences in deposit of 3–6%, but the deposit in the centre was about 5% lower than that on the periphery. With further experience it is hoped to improve the distribution.

In the first replication tests, 1 ml. doses of P31 were sprayed through the nozzle, and the deposits on a cover-slip 2 × $1\frac{1}{4}$ in. found by weighing. It should be emphasized that in order to obtain good replica-

tion of deposit the nozzle must be kept free of obstruction: for instance, a single, minute, cellulose fibre in the nozzle is sufficient to upset the replication. The P31 was therefore filtered through both filter paper and sintered glass.

Table 1 shows the results of the first replication tests.

The data of Table 1 indicated that replication was satisfactory at settings of 0–1 turn and at pressures of 15–20 cm. of mercury, though replication was better at 20 cm. than at 15 cm. pressure. The level of deposit rose with coarser setting, but this effect was less marked at the higher air pressure. Why the time

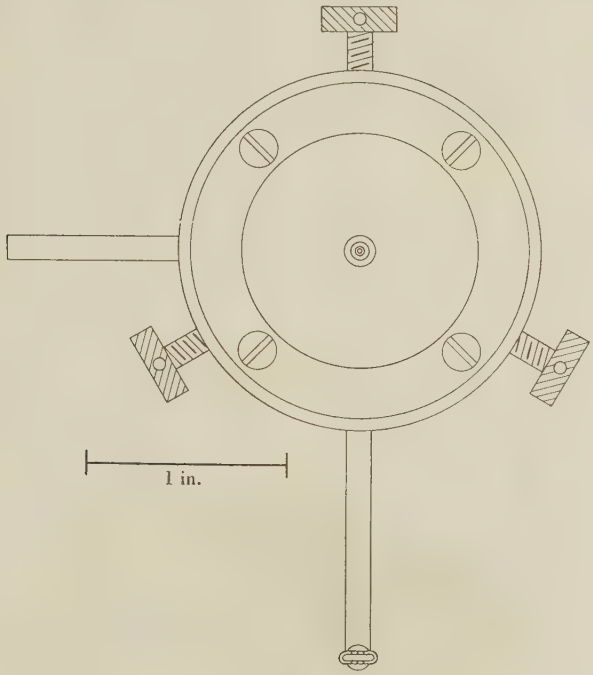


Fig. 2. Plan of atomizing nozzle P.I.L. No. AN2.

TABLE 1. *Replication tests using atomizing nozzle P.I.L. No. AN2*

Air pressure (cm. of mercury)	Nozzle setting (turn)	Deposit (mg.)	Time to spray 1 ml. (sec.)
15	0	11·7	35
		12·4	
		12·1	
	½	13·0	39
		13·3	
		12·9	
	1	14·6	34
		15·0	
		13·9	
20	0	10·5	29
		10·4	
		10·3	
	½	10·8	33
		10·5	
		10·7	
	1	11·6	27
		11·8	
		11·8	

to spray should have been longer at ½ turn than at 0 or 1 turn cannot at present be explained.

As replication was better at 20 cm. of mercury, a further replication test was carried out at this pressure and gave the following results:

Dose	1·0 ml.
No. of sprayings	10
Mean deposit on cover-slip	10·72 mg.
Standard deviation of mean deposit	0·125 mg.

Replication at this level of deposit (0·631 mg./sq.cm.) was therefore good. Another test was carried out using the same pressure to discover whether the variability of the deposit increases with increasing deposit. The results of two sets of sprayings were as follows:

Dose (ml.)	0·5	2·0
No. of sprayings	5	5
Mean deposit on cover-slip (mg.)	4·76	21·12
Standard deviation of mean deposit (mg.)	0·174	0·141

Whereas the mean deposit in the second set of sprayings was over four times that in the first, the

standard deviations were nearly the same; and both were of the same order as that in the previous replication test, so that the variability of deposit appears to be independent of the level of deposit, at least within the range of deposit tested (0·3–1·3 mg./sq.cm.). Although the ratio of doses was 1 : 4, the ratio of the corresponding mean deposits was 1 : 4·44. The deposit is therefore not directly proportional to the dose, but the relation between these quantities was not investigated under the conditions of the replication test, because it would be more useful to do this under the spraying conditions used in experimental work on insecticides.

Discussion

While the design of AN2 has proved generally satisfactory, there is still room for improvement. The optimum sizes for the cones could be determined, and rotation of the liquid feed stem relative to the body could be prevented by a minor alteration in the design. Centring of the cones should be easier if the three centring screws were set at angles of 90°, 135°, and 135°. Centring would then be carried out by readily calculated adjustments of the two screws with an angle of 90° between them, the third screw being tightened when centring was complete.*

In work on insecticidal sprays, the standard deviation of deposit is usually a better measure of the performance of the nozzle than standard error, because, in general, the variation between individual deposits is more important than the difference between the means of sets of deposits. The approximate constancy of the standard deviation of the deposit given by AN2 implies that the relative variability of deposit decreases with increasing deposit. The combined standard deviation of deposit for all replication tests carried out with a nozzle setting of ½-turn and an air pressure of 20 cm. of mercury was 0·0088 mg./sq.cm. The standard error of this combined standard deviation was 0·0014 mg./sq.cm.

Replication tests have been carried out on a nozzle of Potter's (1941) design by the same technique as in tests on AN2. In a typical series of 11 sprayings it gave a standard deviation of 0·065 mg./sq.cm., i.e. seven times that given by AN2. Potter (1941) records the replication given by a nozzle of his design, using a heavy white oil very similar to P31: a standard deviation of 0·073 mg./sq.cm. was calculated from Potter's figures. The two standard deviations agree well, though Potter used a different technique in his tests. Considerable experience in the use of both types of nozzle in bio-assay work with insecticides in a heavy oil base, e.g. P31, has shown that AN2 has a superior performance, especially in replication, and can be more easily and precisely adjusted.

This work has been carried out as part of the programme of research of the Pest Infestation Laboratory, and this account is published by permission of the Department of Scientific and Industrial Research. I am much indebted to Mr Rockliffe of the Building Research Station, Watford, with whom most helpful discussions were held, and who was responsible for the construction of the new nozzles.

* Since this paper went to press these improvements have been successfully incorporated in another nozzle, P.I.L. No. AN3, except that the cone sizes were the same as in AN2.

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The provision of supplementary food to hive bees

By C. G. Butler, *Bee Department, Rothamsted Experimental Station, Harpenden, Herts*

Sucrose syrup is less satisfactory than honey as a winter and spring food for bees: a mixture of approximately equal parts of honey and syrup is of almost the same value as honey alone.

Feeding confined to early autumn induced the strongest spring development of colonies; syrup feeding in the spring may retard colony development, and food supplied at this time is apparently wasted. Feeding with syrup and pollen is advantageous only when a colony is lacking in stores of carbohydrate and protein.

A total of four British Standard brood combs full of pollen provides sufficient protein for a colony on B.S. equipment from autumn until April: the best results were obtained by providing colonies on eleven B.S. combs, in early autumn, with 35–40 lb. of honey, or honey and concentrated sucrose syrup, and four brood combs full of pollen.

Introduction

For many years, beekeepers have discussed the problem of choosing the most satisfactory times, quantities and materials for giving supplementary food to bees. The equal merit of sucrose from beet and from the sugar cane was established by Morland (1929). The possibility of stimulating colonies of bees to rear brood more rapidly than they would normally do, by feeding syrup, or pollen supplements, in spring remains a matter of controversy among beekeepers.

To determine the value of accessory spring and autumn feeding in the Harpenden district, a series of experiments was maintained at Rothamsted from 1939 to 1944. The data from these experiments is analysed and discussed below.

Honey compared with sucrose syrup as a winter and spring food for bees

Early in September 1939, thirty colonies of bees of approximately equal strength, headed by hybrid Italian queens, were selected. These colonies were all housed in 'National' single-walled hives and the queens and their workers had free access to eleven B.S. worker brood combs and eleven shallow combs. The colonies were divided into groups A, B and C, each of ten colonies selected at random. Those in group A were left with approximately 35 lb. of honey and given no sugar or other artificial food during autumn, winter or spring. The colonies in group B were each left with 15 lb. honey and given 20 pt. of concentrated syrup* in the early autumn only. Group C colonies were left with no honey but each was fed with 35 pt. of concentrated syrup in the autumn only. All thirty experimental colonies had an abundant supply of natural pollen.

In mid-April all colonies were examined and their strength determined. Two measures of colony strength were employed.† The first was an estimate of the number of standard brood combs occupied by the bees. For this purpose a shallow comb was taken as being equivalent to half a brood comb. Second, the area of comb occupied by brood of all ages, including eggs, was measured. The brood area of any comb was assumed to be elliptical and its area estimated from measurements of the two axes. Table 1 gives a summary of the results obtained.

It is clear, therefore, that there is no significant difference between groups A and B but there are significant differences between either group A or group B, and group C; the last named group of colonies being weaker after the winter than colonies in either group A or B. It appears from this experiment, therefore, that syrup alone is not such a satisfactory winter carbohydrate for bees as either honey alone or a mixture of approximately equal proportions of honey and syrup.

Table 1. *Honey versus sugar syrup as winter food for bees*

	A Honey only	B Honey + syrup	C Syrup only	S.E.
No. of combs occupied by bees in mid-April (means)	10·2	10·2	8·1	± 1·18
Brood area (sq.cm.) in mid-April	5143	5094	3096	±380

* 2 lb. white granulated sugar (sucrose) in 1 pt. water is 'concentrated' syrup: 1 lb. sucrose in 1 pt. water is 'dilute' syrup, unless otherwise stated.

† These methods of determining and expressing colony strength are employed throughout the present paper.

Autumn compared with spring or autumn plus spring feeding

In September 1940, twenty-five colonies of Italian bees of approximately equal strength, housed in 'National' single-walled hives, were divided at random into five groups of five colonies per group. All the colonies had abundant stores of pollen. The treatment of the different groups is shown in Table 2, together with the results recorded in mid-April.

During the following autumn a further experiment was laid out: thirty colonies of Italian bees of approximately equal strength were divided at random into two equal groups A and B. The colonies in group A were wintered with approximately 15 lb. of honey supplemented with 20 pt. of concentrated syrup fed in the autumn. No spring or winter feeding was carried out. The colonies comprising group B were each wintered with approximately 15 lb. of honey. No supplement was fed until the spring when 20 pt. of concentrated syrup were given. All colonies had abundant stores of pollen.

At the end of April the strength of the colonies in each of these was measured as before. A summary of the results is given in Table 3.

From Table 3 it is seen that the treatment given to the colonies in group A clearly produced the better results, both in number of combs covered by bees and brood area. Table 2 shows that the treatments applied respectively to groups A and B resulted in stronger colonies than the treatments applied to the colonies comprising groups C, D or E. Thus both experiments show the apparent harmful effect of supplementing the honey stores by feeding syrup, either concentrated or dilute, in the spring, much better results being obtained by the provision of comparable stores in the autumn.

The reputed stimulative effect of spring feeding

Data obtained during two previous winters (1940–1, 1941–2) showed that better results were obtained by completing all necessary feeding in the autumn and omitting spring feeding altogether, but as so-called 'spring stimulation', which entails feeding the colonies concerned with syrup in the spring, is such a widespread practice in this country it was felt that the supposed benefits resulting from this treatment required further investigation. In the previous two experiments the colonies destined to be fed in the spring had all been left with only about 15 lb. of carbohydrate stores in the autumn, the balance of the 35 lb. necessary to maintain the colonies until the end of the following April being given in the spring. In the present investigation, fifty-seven colonies of bees divided between two apiaries and headed by young Italian queens were used. All these colonies were wintered with 35 lb. of stores (honey plus syrup) and abundant pollen reserves. The colonies were divided into a number of groups for treatment, as shown in table 4, no treatment being applied until 2 Mar. 1945.

At the end of April each of the experimental colonies was checked to determine the number of combs covered by the bees, the brood area, and the carbohydrate stores remaining. The results are given in Table 4.

The records summarized in Table 4 show no consistent differences between rapid and slow feeding nor between syrup with and without soya bean candy. The reduction in the area of the brood for the colonies fed with concentrated syrup as compared with the controls is just significant, and the intermediate value for the colonies fed with dilute syrup

Table 2. *Effects of autumn versus spring, or autumn plus spring, feeding*

	A 35 lb. honey. No winter or spring feeding	B 15 lb. honey +20 pt. conc. syrup in autumn. No spring or winter feeding	C 15 lb. honey +20 pt. conc. syrup in spring. No autumn or winter feeding	D 15 lb. honey +30 pt. dilute syrup in spring. No autumn or winter feeding	E 15 lb. honey + 6 lb. candy from mid-Feb. onwards and 14 pt. conc. syrup in spring. No autumn feeding	S.E.
Combs occupied by bees in mid-April (means)	9·2	9·2	7·2	8·0	7·4	± 0·53
Mean brood area (sq.cm.) in mid-April	5090	4910	3520	3990	3730	±330

Table 3. *Results from autumn versus spring feeding*

	A Colonies fed in autumn	B Colonies fed in spring	S.E.
Combs occupied by bees at end of April (means)	9·4	7·9	± 0·3
Mean brood area (sq.cm.) at end of April	4880	4170	±150

supports this indication. Similar differences occur in the mean numbers of brood combs covered by the bees, but further evidence is necessary before this effect can be considered definitely established.

Table 4 also shows that the residual stores of the control colonies, which were not fed in the spring, were about the same as those of the experimental colonies receiving a considerable amount of extra syrup in the spring.

Thus the results of the 1944–5 experiment supported those previously obtained in showing that spring feeding leads neither to stimulation of the colonies nor to an increased rate of colony development.

group B had four B.S. brood combs of pollen each, and the colonies in group C had two B.S. combs of pollen each. Most of the pollen reserves in the experimental colonies were sealed over.

Towards the end of April 1944 the number of combs covered by the bees and the amount of brood in each colony was estimated.

From the data in Table 5 it will be seen that those colonies left in the autumn with only two combs of pollen were definitely inferior in strength in the following spring to those left with four to six combs of pollen, the two last named being equal. It would appear, therefore, that under the conditions of this

TABLE 4. *Effect of spring feeding*

(Mean results at end of April 1945)

R=rapid feeder. *S*=slow bottle feeder.

Group		1	2	3	4	5
Weekly spring feeding per colony		3 pt. conc. syrup	6 pt. dil. syrup	2 B.S. combs soya bean candy*+3 pt. conc. syrup	2 B.S. combs soya bean candy*+6 pt. dil. syrup	None
No. of colonies	*R*	4	5	7	7	10
	S	5	4	6	5	
Combs covered by bees (±2·2)	*R*	7·5	12·6	8·1	11·3	12·1± 1·6
	S	9·8	9·2	9·8	12·4	
Brood area (sq.cm.) (±1220)	*R*	4290	6480	3760	6160	6540 ±870
	S	4070	4890	5150	6130	
Carbohydrate stores (lb.) (±3·6)	*R*	14·0	16·2	16·0	14·7	15·0± 2·5
	S	16·0	14·5	12·6	15·6	

* Prepared by kneading fresh soya-bean flour (Expeller processed) with sufficient concentrated sugar syrup to make a fairly dry crumbly paste. A trace of thymol was added to discourage fungal growth and the paste was pressed into the cells on both sides of empty brood combs, two such combs being supplied per colony, one on each side of the brood nest.

TABLE 5. *Effect of wintering colonies of bees with different quantities of stored pollen*

	A. 6 combs of pollen	B. 4 combs of pollen	C. 2 combs of pollen	S.E.
No. of combs occupied by bees at end-April 1944 (means)	9·8	9·8	7·2	± 0·52
Brood area (sq.cm.) at end of April 1944	4990	5040	3810	±170

OPTIMUM QUANTITY OF POLLEN REQUIRED FOR WINTERING

During September 1943, fifteen colonies of Italian bees in W.B.C. hives were divided into three groups with five colonies in each. The queen and worker bees in each colony had free access to ten B.S. brood combs and ten B.S. shallow combs, and the colonies in each group were wintered with approximately 35 lb. of honey plus syrup each, all the feeding necessary being completed by mid-September. No winter or spring carbohydrate feeding was practised. In addition each of the five colonies comprising group A had six B.S. brood combs full of natural pollen left to them in the autumn, the colonies in

experiment about four B.S. brood combs full of pollen were necessary for successful wintering and spring build-up. This conclusion is in close agreement with the findings of Jamieson (1945), who recorded that in Canada 600 sq.in. or three B.S. brood combs of pollen were required for successful wintering.

I should like to take this opportunity of expressing my thanks to members of the Statistical Department at Rothamsted, particularly to Mr D. J. Finney, lately of that Department, and to my colleagues in the Bee Department who have assisted me with this work.

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Further investigations on the value of electric heating of beehives

By C. G. Butler, *Bee Department, Rothamsted Experimental Station, Harpenden, Herts*

A set of twenty-four colonies of honeybees was divided into three groups, two of which received different intensities of artificial heat, applied by means of specially designed heating frames placed in the middle of the brood chambers, from 2 Jan. to 16 Mar. 1942. Heating the colonies reduced the area of brood present at the beginning of May without noticeably affecting the consumption of carbohydrate stores and thus had an adverse effect on the colonies. This conclusion is in agreement with that reached as a result of earlier work (Butler & Cockbill, 1942.)

Colonies in hives fitted with top entrances were no stronger in the spring than colonies in hives with bottom entrances, nor did they show any significant difference in store consumption.

No significant brood chamber temperature differences were found in hives fitted with top entrances as compared with control hives which had normal bottom entrances.

During the period when heat was applied to the colonies by means of the heating frames the minimum brood chamber temperature was significantly higher in the heated hives than in the control, unheated, hives, this being especially marked in the case of the hives fitted with 6 W. heaters.

Throughout the experiment the minimum brood chamber temperature of all the hives showed a close dependence on screen temperature. During the preliminary period before the heat was turned on a change of 1° C. screen temperature was associated with change of 0·92° C. in the minimum brood chamber temperature in hives with top entrances, and a change of 0·86° C. in hives with bottom entrances. After the heat was turned on the corresponding figures were 0·84° C. for top entrance hives and 0·67° C. for the bottom entrance hives.

Introduction

The results of certain preliminary experiments conducted in the Home Apiary of Rothamsted Experimental Station during the winter 1940–1 with an electrical heating device (Wedmore, 1941) designed for use in beehives have already been published (Butler & Cockbill, 1942.)

The conclusion was reached from these preliminary trials that the general effect of heating the colonies of bees had been to reduce the area of brood present in these colonies in mid-April and to increase their consumption of carbohydrate stores, in direct contradiction of any belief in a beneficial effect on the colonies. The heating device employed in these preliminary experiments was in the form of a special crown-board which was heated from above by means of two 15 W. 230 V. lamps run in series and giving an output of 9–10 W., and placed immediately above the brood chamber. The heaters were well lagged above with dry sawdust, and hot spots due to the heating elements were eliminated by the use of a metal plate to distribute the heat combined with a small baffle plate placed immediately beneath each heating unit.

Despite the results obtained in these preliminary trials it was decided to conduct a further series of tests, during the winter 1941–2, in collaboration as before with the Electrical Research Association.

Methods

It seems probable that the ideal position in which to apply artificial heat to a colony of bees during the winter is within the cluster of bees itself, but this presents the difficulty that the bees must be free to move anywhere within the brood chamber and must not be impeded in their movements by the heater, nor must any parts of the heating unit with which the bees can come into contact present any inconveniently hot spots which would disturb them. A heating unit satisfying these conditions, and consisting of wooden bars on which the insulated heating element was non-inductively wound and between which the bees could pass freely, or upon which they could cluster, built into a British Standard brood frame was designed by Mr E. B. Wedmore of the Electrical Research Association for use in this second series of experiments. Sixteen of these heating frames were prepared, half being wired for

3 W. and the other half for 6 W. A further eight dummy heating/frames, similar to the others but unwired were made up for use in the control colonies.

Twenty-four colonies of bees housed in 'National' single-walled hives and headed by young Italian queens of the same age and strain were employed. Twelve of the hives with normal bottom entrances housing these colonies were arranged as follows commencing from the bottom of the hive: floorboard with full entrance protected against the entry of mice with a piece of perforated zinc having a flap 2 in. long by ¼ in. high cut in the centre and turned outwards and upwards to serve as an entrance and exit for the bees; empty shallow super; brood chamber with brood combs; wooden crownboard with a 2 in. diam. feedhole cut in the centre and covered with a piece of perforated zinc; empty shallow super; empty brood chamber; deep roof with two 1 in. diam. ventilation holes in two opposite sides. The remaining twelve hives had top entrances, i.e. entrances above the brood chamber, and were arranged as follows: floorboard with entrance completely blocked with a tightly fitting piece of wood; empty shallow super; brood chamber with brood combs; wooden crownboard with 2 in. diam. feedhole cut in the centre and left uncovered; empty shallow super with one side raised ¼ in. from the bottom throughout its length so as to provide a top entrance the full width of the hive through which the bees could pass after coming up from the brood chamber through the open feedhole; empty brood chamber; deep roof with ventilation holes. No packing was given to any of the colonies in these twenty-four hives.

Four of the colonies housed in hives fitted with top entrances and four colonies in hives with normal bottom entrances were selected as controls, and a dummy heating frame was placed in the centre of the brood chamber of each of these colonies with five brood combs on either side of it. Another four colonies in hives with top entrances, and four in hives with bottom entrances, were each supplied with 3 W. heater frames placed in the centre of the brood chamber with five combs on either side. The remaining eight colonies, four with top entrance and four with bottom entrance hives, each had a 6 W. heater frame placed in the centre of the brood chamber. The allocation of treatment to the experimental colonies was carried out at random.

Each of the twenty-four experimental colonies was left with 25 lb. of honey and this was supplemented in each case by feeding 10 lb. of sucrose as a concentrated solution (2 lb. sugar per pint of water), this feeding being completed by the end of September 1941 after the heating frames had been put in place. Plentiful supplies of pollen were also present in each colony, and the strength of all colonies both in bees and brood was equalized as nearly as possible.

On 1 Mar. 1942 two from each of the three groups of colonies in hives fitted with bottom entrances were given 4 pt. of very dilute syrup (¼ lb. sugar per 2 pt. water) fed in rapid feeders within the hives. When this syrup had been taken the feeders on these colonies were kept constantly supplied with rainwater.

Each of the twenty-four hives was fitted with two thermocouples, one on each of the lateral walls of the brood chamber, the combs in which were arranged at right angles to the hive entrance. No attempt was made to measure the temperature within the cluster, as had been done in the previous experiments, these two thermocouples merely being situated so as to give an approximation to the hive temperature at a point well away from the bees. Temperature readings were taken at 09.00 hours G.M.T. every 2 or 3 days from 27 Oct. 1941 until the end of the experiment, and the lower reading of the two thermocouples in each hive was taken as the representative figure on each occasion. The two readings were usually fairly close to each other, but occasionally one or other was much increased owing to the proximity of the cluster of bees.

Results and conclusions

Between 1 and 4 May 1942 the twenty-four colonies were examined and, as in 1941, measurements of the total area of brood in each were made. Estimates of the number of combs covered by bees and total amount of carbohydrate stores remaining in each case were also made at the time of this examination. Two colonies, both in hives with bottom entrances, had to be rejected, as the bees in one were dead and the other had a 'drone-laying' queen. Both were in the 3 W. heating group, one being a colony to which additional very dilute syrup had been fed.

The brood area per colony, as shown in Table 1, was very much greater than in the 1940–1 experiment, but again showed very high variability. The apparent reduction in brood area in the 3 W. group of colonies as compared with the unheated colonies is well within the limits of experimental error, and as it is not supported by the results obtained with the 6 W. group of colonies little importance need be attached to the result. Indeed, with standard errors as large as in this experiment only differences between treatments of the order of 1000 sq.cm. could be satisfactorily shown by means of eight colonies of bees. This experiment and that of 1940–1 agree in the suggestion that in order to demonstrate clearly differences in brood area as small as 10% approximately 80–100 colonies would be necessary in each treatment group.

The colonies subjected to the higher level of heating used appreciably less carbohydrate stores than the others, the mean difference being about

2½ lb. per colony. No satisfactory explanation can be given as the colonies with 3 W. heaters actually used slightly more stores than the control, unheated, colonies. No significance can be attached to the small difference between the numbers of brood frames covered by the adult bees for the three treatments.

Table 2 shows the mean values of the brood area, consumption of stores and number of brood frames of adult bees for the colonies housed in hives with top and bottom entrances, and for the colonies in bottom entrance hives which were fed with additional very dilute syrup followed by water in the early spring. There are clearly no well marked differences, and it appears that the two measurements of colony strength, and of the consumption of stores, have been spite of the increase in precision, none of the differences can be considered significant, though it is noticeable that the colonies housed in hives with 6 W. heating frames had a greater adjusted mean brood area than either of the groups of colonies in hives with 3 W. heaters or no artificial heat.

In view of the failure to find any significant effect of the heating treatments or of the position of the hive entrance on colony strength at the May examination, there is no great interest in studying the temperature records, particularly as the situation of the thermocouples was such as to provide a purely physical rather than a biological measurement.

During the period when heat was applied to the colonies by means of the heating frames the minimum temperature was significantly higher in the heated

TABLE 1. *Effect of heating on colony strength and consumption of carbohydrate stores as shown at the May examination*

Mean values per colony.

Heat supplied (W.)	Brood area (sq.cm.)	Consumption of carbohydrate stores (lb.)	No. brood frames of adult bees
0	2660 ± 320	32·3 ± 0·74	5·5 ± 0·5
3	2080 ± 370	33·3 ± 0·86	4·7 ± 0·6
6	2500 ± 320	30·1 ± 0·74	5·0 ± 0·5
Mean	2420	31·9	5·1

TABLE 2. *Effect of position of hive entrance and of additional very dilute syrup fed in the early spring on colony strength as shown at the May examination*

	Brood area (sq.cm.) Mean per colony	Consumption of carbohydrate stores (lb.)	No. of brood frames of adult bees
Top entrance	2440 ± 260	31·8 ± 0·61	5·3 ± 0·4
Bottom entrance	2120 ± 410	32·6 ± 0·94	4·7 ± 0·6
Bottom entrance + very dilute syrup	2660 ± 410	31·3 ± 0·94	5·0 ± 0·6
Mean	2420	31·9	5·1

insufficiently affected by the position of the hive entrance and by the additional supply of syrup for the effects to be detected.

More detailed analysis of the brood areas showed them to be associated with the hive temperatures recorded in spite of the temperature inside the brood chamber being taken at a point as distant from the cluster as possible. There was a considerable degree of variation shown by these hive temperatures even during the preliminary part of the experiment before the heating frames within the hives were turned on. Brood chamber temperatures differed by as much as 1° C. over the period 5 Nov. to 31 Dec. 1941, and greater accuracy in the final comparisons can be obtained by adjusting the mean brood areas for these initial temperature conditions. Adjusted figures for levels of heating are shown in Table 3, and for the position of the hive entrance in Table 4. It will be seen from these tables that in

TABLE 3. *Effect of heating on brood area adjusted for initial hive temperature differences*

Heat supplied (W.)	Mean brood area (sq.cm.)
0	2190 ± 300
3	2210 ± 290
6	2850 ± 280
Mean	2420

TABLE 4. *Effect of position of entrance and of additional very dilute syrup fed in the early spring on brood area adjusted for initial hive temperature differences*

	Mean brood area (sq.cm.)
Top entrance	2300 ± 210
Bottom entrance	2690 ± 370
Bottom entrance + very dilute syrup	2370 ± 330
Mean	2420

hives than in the control, unheated hives, especially in the hives fitted with 6 W. heaters. The mean minimum hive temperatures for the period 2 Jan. to 16 Mar. 1942 are shown in Table 5. All four of the hives fitted with 3 W. heating frames and having bottom entrances had unusually low temperatures. No explanation of this can be offered, and it seems that the mean value for this level of heating is biased by this anomalous behaviour. No significant hive temperature differences were found in hives fitted with top entrances as compared with those with bottom entrances.

Table 5. *Mean minimum brood chamber temperature in ° C. recorded during the period 2 Jan. to 16 Mar. 1942*

	Heat supplied (W.)			
	0	3	6	Mean
Top entrance	1·2	1·8	2·2	1·7
Bottom entrance	1·2	0·9	2·5	1·5
Mean	1·2	1·4	2·3	1·6

Mean screen temperature = −0·4° C.

Throughout the experiment the minimum brood chamber temperature showed a close dependence on the screen temperature. In the brood chambers of those hives that were fitted with top entrances a change of 1° C. in screen temperature was on an average associated with a change of 0·92° C. in the minimum brood chamber temperature during the preliminary period, 5 Nov.–31 Dec. 1941, before the heating frames were turned on, and 0·84° C. during the heating period, 2 Jan.–16 Mar. 1942. The corresponding figures for those hives that were fitted with bottom entrances were 0·86° C. for the first period and 0·67° C. for the second. During both periods the value for the hives fitted with top entrances was significantly higher than for the hives with bottom entrances, an effect which probably means nothing more than that the points at which the thermocouples were placed within the brood chamber were such as to be more susceptible to changes in outside temperature in hives with top entrances than in hives with bottom entrances.

The dependence of brood chamber temperature on screen temperature was not investigated for the three levels of heating separately, but it may be noted that on the average of all hives this dependence was a little less close in the period after the heat was turned on than in the previous, unheated period. Throughout the whole course of the experiment this dependence of brood chamber temperature on screen temperature was substantially more marked in the 1940–1 experiment, though the significance of the difference may be doubted.

I wish to take this opportunity of thanking Mr E. B. Wedmore, of the Electrical Research Association, for his interest in this work and for designing the frame heating units. My thanks are also especially due to Mr D. J. Finney, lately of the Rothamsted Statistical Department, for considerable assistance in the analysis of the data obtained, and also to my colleagues in the Bee Department.

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The ecology and function of Protozoa in sewage purification

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(With 3 Text-figures)

Seasonal recording of the Protozoa in various sewage disposal units has shown that, together with changes correlated with the degree of purification of the sewage, fluctuations occur as a result of the interplay of seasonal non-specific factors and local specific factors. The incidence and abundance of Protozoa in each disposal plant would seem to be controlled by sewage 'strength' and characteristic local drainage. Fluctuations of the population, however, are limited by different factors in different seasons.

In the bacteria bed investigations a 'weak' sewage shows a decrease of a generally rich fauna during the periods of sloughing, a 'strong' acid sewage during sloughing shows an increase in an otherwise restricted fauna and where the sewage is medium in 'strength' and supports an abundant insect population the fluctuations of the Protozoa are dominated by the more active insect populations of summer and by temperature in winter. In this last-named environment a significant inverse correlation between ciliate and insect frequency throughout the summer indicates the thoroughness with which the insect larvae devour the film; this may explain the summer reductions in certain groups of Protozoa.

In activated sludge channels conditions favour a uniform population which may vary in composition according to the purity of the sewage. Flocculation is most pronounced when protozoan populations comprise a restricted variety of ciliates and would seem to depend on a protozoan fauna since cultures of bacteria obtained from sewage did not flocculate unless Protozoa, free from or contaminated with bacteria, were added.

The protozoan populations of the two systems of disposal are compared in discussion.

Introduction

Recent investigations upon the fauna of sewage disposal plants have emphasized its importance in relation to the efficiency of the purification process through its disintegrative action upon the complex organic matter passing through the system. The fauna embraces many invertebrate groups (Lloyd *et al.* 1940) and the insect and enchytraeid populations have been studied respectively by Lloyd (1937, 1941, 1943) and Reynoldson (1939). Purification of sewage results from biological activity in the presence of oxygen and this investigation deals with the role of the protozoan fauna in the two main methods of disposal now employed—the bacteria bed process, in which the sewage is aerated during distribution as a film over stones, and the activated sludge process, where sewage in bulk is aerated in channels by rotating paddles or compressed air.

Records exist of the Protozoa associated with bacteria beds (Johnson, 1914; Crozier & Harris, 1923; Hausman, 1923; Lackey, 1924, 1925; Cutler *et al.* 1932; and Barker, 1942*a*, 1943*b*), and those in activated sludge have been grouped according to the efficiency of the sludge they inhabit (Lockett, 1928; Barritt, 1940). Other studies have indicated the value of the Protozoa in purification and their relation to bacterial activity (Viehl, 1937; Lockett, 1928; Meiklejohn, 1932; Pillai & Subrahmanyan, 1942, 1944; Jenkins, 1942; Hardin, 1943; Watson, 1943, 1945).

The Protozoa, apart from seasonal change, are influenced by the fluctuating conditions in the system and it is the purpose of these investigations, by surveying and comparing the protozoan faunas of various systems throughout the year, to determine how these faunas are related to the various environmental factors and to note any evidence indicating their influence on the purification process.

Material and method

Observations upon the Protozoa were taken in the first instance at Knostrop, Leeds, but later extended to Oulton, Bradford, Huddersfield and Rodley where sewage and media were of different composition. Thus a basis for comparison was obtained. Similar records were taken at Leeds, Oulton and Bradford throughout the year to determine the range of seasonal change and its relation to local conditions. These data were correlated with analyses of effluent sewages to form a comprehensive survey of the fauna in relation to purification.

The system at Knostrop is a typical arrangement with sedimentation tanks, travelling distributors working over 6 ft. deep gravel bacteria beds kept open by the vigorous scouring activities of an association of insects and worms (Parkinson & Bell,

1919; Lloyd, 1937; Reynoldson, 1939), and humus settlement tanks which, like the sedimentation tanks, are emptied and cleaned weekly. The sewage treated is mixed in character, medium in 'strength' and neutral to alkaline in reaction.

The Oulton beds are circular, with gravel below and clinker above, and, although the macrofauna is not so dense, they remain open as the sewage is 'weak', being mainly domestic with only 10–15% of brewery waste. The humus tanks are unusual being 26 ft. deep and never emptied, the humus being syphoned off at intervals.

At Esholt, Bradford, the beds have a medium of small coal and are dosed at a slow rate with a 'strong' sewage about half of which comes from wool scouring plants. The acid, which is added to facilitate sedimentation and grease extraction, produces a *p*H of 3–4 and this, together with the complex sewage, favours a heavy development of fungi in the beds. The rather sparse macrofauna of these beds cannot fully cope with this fungal growth and hence the beds become more heavily loaded with solid than those of Knostrop or Oulton. The humus tanks were not operating during these observations but records from the bed effluent channels afforded equivalent results (Barker, 1942*a*).

Methods of assessing the organisms present in the various environments have been previously described (Barker, 1943*a*).

Seasonal observations were based on film and volume samples taken from both the beds and humus tanks every fortnight. These samples were assessed immediately on return to the laboratory.

In the laboratory, samples of sewage were cultured under varying conditions of temperature, reaction and aeration to check observations of the influence of these conditions in the field. In addition, a model of the activated sludge system, treating Knostrop sewage, was set up in the laboratory to compare the influence of the two systems of purification upon the composition of the fauna.

General account

The genera of Protozoa in the sewage at Knostrop have been previously recorded and grouped according to their environment in the plant (Barker, 1942*a*). Further, the individual species at Knostrop, Oulton and Bradford and those associated with activated sludge have been listed and their individual abundance and seasonal preferences noted (Barker, 1943*b*). The following observations, from detailed analyses of the records compiled in 1940–2, are concerned mainly with the seasonal and general changes of the protozoan populations.

In spite of the varying conditions, a number of organisms were common to all the localities. These organisms include: Rhizopoda—*Amoeba limax* Duj., *A. actinophora* Auerbach, *Vahlkampfia limax* Duj., *V. guttula* Duj., *Arcella vulgaris* Ehr., *Trinema lineare* Pen., and *Actinophrys sol* Ehr.; Mastigophora—*Mastigamoeba* sp., *Oikomonas termo* Clark, *O. steinii* Kent, *Monas fluida* Duj., *M. minima* Meyer, *Pleuromonas jaculans* Perty, *Bodo lens* Klebs, *B. caudatus* Duj., *Cercobodo ovata* Lem., *C. longicauda* Senn., *Trepomonas agilis* Duj., and *Notosolenus orbicularis* Stokes; Ciliophora—*Chilodon* sp., *Colpidium colpoda* Stein, *Trichoda pura* Ehr., *Urotricha farcta* Cl. and L., *Uronema marinum* Duj., *Cyclidium glaucoma* Ehr., *Cinetochilum margaritaceum* Ehr., *Vorticella* spp., *Carchesium polypinum* Linn., *Opercularia* sp., and *Podophrya fixa* Müll.

The influence of local conditions, e.g. the chemical nature of the sewage, type of medium, local drainage, etc., was therefore reflected chiefly in quantitative and qualitative variations of the fauna as shown by the presence or absence of a limited number of species and in the population changes during the year and during passage through the plant.

A. *The sedimentation tanks*

The rapid changes in the chemical constitution of the crude sewage in this environment dominate the seasonal environmental changes so that the incidence of organisms fluctuates rapidly within the limits imposed by a constant anaerobic state resulting from the characteristic Biochemical Oxygen Demand of the unpurified sewage. Thus, anaerobes and facultative anaerobes are favoured by the environment though aerobic forms may survive the passage of the tanks as cysts or near the surface. The fauna of the septic tanks at Rodley resembles that of the Knostrop sedimentation tanks in spite of the fact that the liquid is retained much longer in the former.

In view of the rapid passage of the sewage through the plant and the probable facultative nature of most of the organisms encountered in the tanks it is not surprising that many species persist throughout the whole of the purification process.

B. *The bacteria beds*

Conditions in the bacteria beds form a marked contrast to those of the sedimentation tanks since the sewage, in the form of a thin film, is fully aerated and the accumulation of purifying organisms in the zoogleal film covering the medium confers some degree of stabilisation.

The large variety of species in the beds at all times is in marked contrast to the restricted variety in natural water and suggests a low 'selective potential' for any individual protozoan type. This is due, no doubt, to an abundant food supply, for, in such an environment, species, variety and abundance of Protozoa are in direct relation (Crozier, 1923). By averaging the abundance corresponding to a definite number of species throughout the records this relationship has, in general, been confirmed (Table 1).

The localities, however, vary slightly in this respect, for while Oulton and Bradford generally conform to this relationship, rhizopods alone show this trend at Knostrop, whilst flagellates show a complete reversal due possibly to the peculiar conditions in this bed.

Although all in the Aire valley and only a few miles apart, these localities show the influence of specific conditions in the presence or absence of certain elements of the fauna. Species characteristic of Oulton are *Centropyxis aculeata* Stein, *Cyphoderia ampulla* Leidy, *Codosiga botrytis* Ehr., and *Platycola decumbens* Ehr. At Knostrop, *Paramoecium bursaria* Focke, *Vaginicola tincta* Ehr., *Spirostomum teres* Cl. & L., and *Stentor roeselii* Ehr. are common though they have not been found at either Oulton or Bradford. Esholt (Bradford) has a more restricted fauna and while possessing no characteristic species it lacks *Amoeba proteus* Leidy, *A. villosa* Wallick, *Amphileptus* spp., *Lionotus* spp., *Chilodon cucullulus* Müll., *Paramoecium caudatum* Ehr., and *Epistylis plicatilis* Ehr., all of which occur with some regularity in the other two localities. (Species noted only in the bed effluent have been regarded as present in the beds though sufficiently scarce or localized to be absent from the bed records.)

In regard to seasonal variations, representatives of both orders of fresh-water rhizopods, the Amoebina and the Conchulina, are found in all bed localities and show definite trends in their distribution. The Amoebina are present throughout the year and show maxima during the winter when the Conchulina are generally scarce or absent. The latter follow the general local trend of population density so that at Oulton they are depressed about September while the beds were cleaner as a result of the autumn slough (p. 318) and at Knostrop all forms are depressed about July, when the sewage tends to stagnate and when the insect larvae populating the beds are increasing (p. 318). At Esholt the period preferred by the Conchulina is generally unfavourable to aerobic Protozoa and only a few of these forms are found near the base of the bed. *Cochliopodium* is present during May and June when conditions are improved by a slough and, in spite of the adverse conditions later, *Trinema* rises to an autumn maximum as at Oulton (Fig. 1).

TABLE 1. *Showing the relationship between abundance and multiplicity of species in the three bed environments*

Oulton bed

Rhizopoda		Mastigophora		Ciliophora	
No. of species	Mean abundance	No. of species	Mean abundance	No. of species	Mean abundance
2 and 3	1290	5, 6 and 7	1010	3, 4 and 5	180
4 and 5	1630	8 and 9	5600	6 and 7	660
6 and 7	3170	10 and 11	7890	8 and 9	680
8 and 9	3040	12 and 13	6530	10 and 11	1400
10	7522	14 and 15	26710	13 and 14	6610

Knostrop bed

Rhizopoda		Mastigophora		Ciliophora	
No. of species	Mean abundance	No. of species	Mean abundance	No. of species	Mean abundance
2	60	0–4	10420	2, 4 and 5	690
3	940	5 and 6	2110	6 and 8	1250
4	2170	7 and 8	1080	9 and 10	1250
5	3230	9 and 10	2460	11 and 12	870
6	1290	12	716	13–15	1660

Bradford bed

Rhizopoda		Mastigophora		Ciliophora	
No. of species	Mean abundance	No. of species	Mean abundance	No. of species	Mean abundance
0	0	2 and 3	79	1 and 2	19
1	6	4	122	3 and 4	62
2	70	5	220	5 and 6	90
3	34	6	320	7	190
5	82	7 and 8	340	9	650

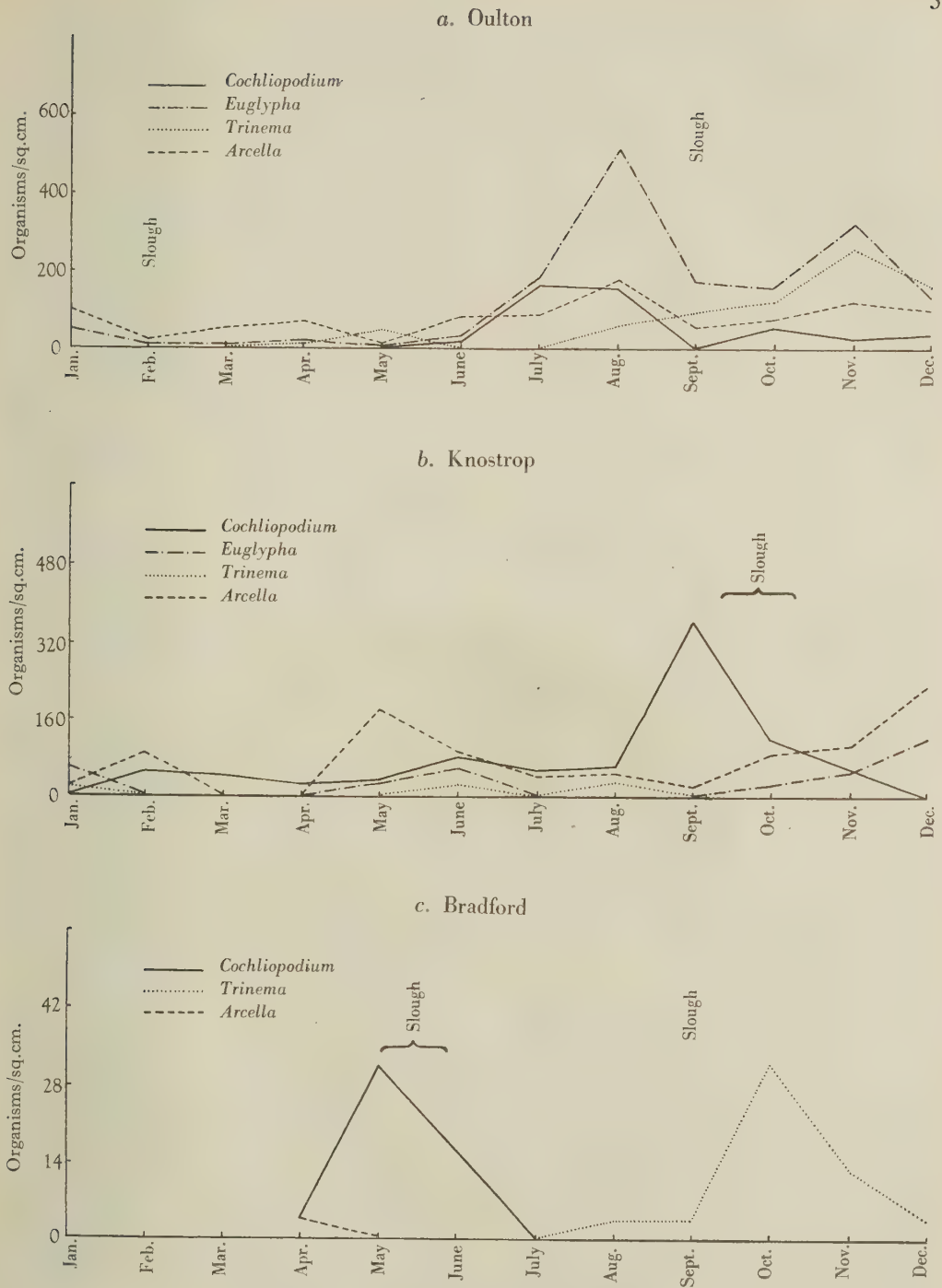


Fig. 1. Showing the seasonal incidence of the Conchulina in the bacteria beds of three sewage disposal plants.

The Mastigophora as a whole have no marked seasonal variations but the commoner *Mastigamoeba*, *Monas*, *Oikomonas* and *Pleuromonas* are generally more abundant in winter, a possible result of insect predatism (p. 323). *Astasia* and *Euglena*, only common at Oulton, prevail during summer as under natural conditions (Wang, 1928; Lackey, 1940). *Trepomonas agilis*, which favours anaerobic conditions (Lackey, 1932), appears at Knostrop in summer and early autumn when the sewage tends to putrefy prior to aeration. *Monosiga steinii*, occurring chiefly at Oulton, prefers autumn conditions.

There is a close relationship between the ciliates, other biotic factors and the physical state of the bed so that this group demonstrates the influence of the combined local conditions better than do either the rhizopods or the flagellates.

Sloughing at Oulton was detected by changes in the zoogleal film and in February and September considerable reductions in the zoogleal matter were noted. At these times both free and sessile ciliates showed a marked reduction, the latter responding more slowly than the former. A more complete reduction of the population and a protracted recovery indicated a more severe slough in spring than in autumn (Fig. 2).

An increase in suspended solids in the effluent at Esholt indicated considerable sloughing in May and June and to a less degree in September and, at these periods, increases in oxygen and ammonia were detected, the sewage was less acid and both nitrites and nitrates were present. Changes in the fauna were most marked during the spring slough when the sessile *Carchesium*, *Opercularia* and *Podophrya*, and the free *Chilodon*, *Trichoda* and *Cinetochilum* were more abundant. *Chilodon* sp., *Cyclidium* and *Urotricha* increased during autumn in spite of the less aerobic conditions prevailing. Thus, in general and in contrast to Oulton, sloughing at Bradford results in an increase in the ciliate population which at other times is restricted and sparse (Fig. 2, Table 3).

Although sloughing occurred at Knostrop, especially during autumn in the year of sampling, no definite response of the ciliate fauna was observed. Fluctuations were in no way consistent though some depression in the fauna in summer suggested that the higher temperatures were in some way effective in controlling general abundance (Fig. 2). A direct temperature effect could have accounted for the depression in certain forms, e.g. *Chilodon* sp. (p. 323), but many forms depressed had shown maxima at this period at Oulton. Further, several species known to be aerophilic were present at this time, e.g. *Opercularia*, *Oxytricha* and *Lionotus*, together with forms capable of surviving more stagnant conditions, e.g. *Cyclidium glaucoma* and *Chilodon cucullulus*, so that stagnation of the sewage was not the controlling influence. It seems therefore that biotic conditions rather than physico-chemical influences were producing these depressions and considerations of the established behaviour of Enchytraeidae (Reynoldson, 1939) and Insecta (Lloyd, 1937, 1941, 1943) suggest possible interrelationships which have not hitherto been considered. The worm population showed seasonal variations in the intensity of their distribution in the beds and hence in their region of greatest activity, but there was no appreciable change in total abundance. On the other hand, marked changes occur in the seasonal insect population curve which displays a series of peaks as species after species (Lloyd, 1937, 1943) and generation after generation (Lloyd, 1941) come to their maxima. The index of total ciliates (estimated from slides 1 ft. below the bed surface) was set against the index of total grazing insects (estimated as the total flies trapped over 1 sq.ft. of bed—taken from Lloyd's records). Peaks of fly output on 14 Mar. and 28 Apr. are each accompanied by a depression of ciliates while depression of flies on 20 June and 4 July, indicating a relative failure of larvae during the preceding fortnight, is accompanied by abundance in ciliates on 20 June. From 1 Aug. to 24 Oct. the peaks of insect and the depression of ciliates keep in exact step and there is a high significant negative correlation ($n=5$, $r=-0{\cdot}90$ and $P=$ less than $0{\cdot}01$). The correspondence breaks down after 21 Nov. and the fluctuations of both bear a more direct relation (Fig. 3, Table 2).

The depth distribution of the various species of Protozoa at Esholt show significant zoning in the testate rhizopods and some ciliates (Table 3). Among the former, *Arcella*, during its short season, is abundant at the base of the bed (see also Cutler *et al.* 1932; Johnson, 1914) and is later replaced by *Trinema*, also most abundant in this region. The ciliates are most abundant in the middle depths through most of the year, but many species tend to accumulate near the base during May. These forms include especially *Opercularia* and *Chilodon* sp. whilst *Carchesium* and *Podophrya* extend the period of descent into June. With the exception of *Chilodon* sp. these ciliates, at this time, reached a significant maximum. Again in September a less well defined majority of ciliates were at the base of the bed. Amoebina and Mastigophora are generally less frequent at the base than in the upper or middle regions and the latter are, in seven of ten months' continuous records, abundant at the surface.

A high protozoan count in the beds is associated with a low count in the humus tank (Table 4) and in conjunction with this, fourteen species out of sixty-eight recorded from the Oulton beds were not observed in the bed effluent and four species in the latter were not seen in bed samples, whereas at Esholt, two species from the bed were not recorded in the effluent and fourteen species from the latter were not recorded from the bed.

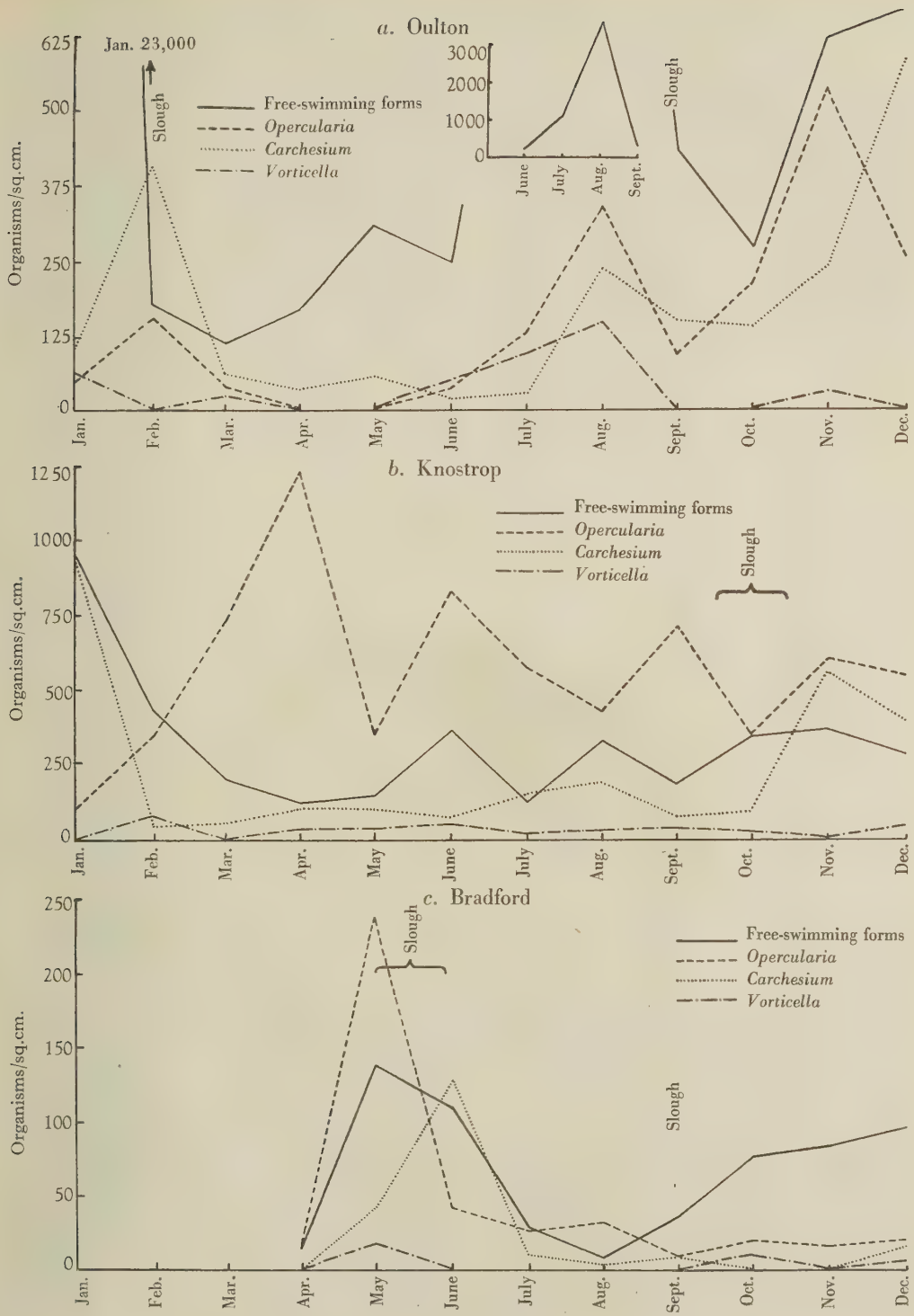


Fig. 2. Showing the seasonal incidence of the Ciliophora in the bacteria beds of three sewage disposal plants.

C. *The humus tanks*

Fluctuations in the humus tanks follow those of the bed very closely, the seasonal succession previously reported (Barker, 1942*a*) following closely the bed succession and being repeated in the humus tank during a second year of observations. The only deviations were an earlier appearance of *Cyclidium*, which had a longer season, and the later appearance of *Paramoecium*, which had a shorter season (Barker, 1942*b*). Depth distributions in the Knostrop humus tanks showed no significant variations but at Oulton there was a significant increase in rhizopods, flagellates (excluding phytomonads) and the appearance of *Colpidium colpoda* at the surface of the sludge suggesting that in this region conditions are similar to those obtaining at the surface of the bed.

D. *Activated sludge*

The fauna of the activated sludge channels at Huddersfield, where the sewage contains a high proportion of chemical waste, was composed of rotifers, nematodes and Protozoa. Flagellates were generally abundant, but rhizopods (*Amoeba limax* and *Vahlkampfia guttula*) and ciliates (*Vorticella*) scarce—a condition indicating inefficient sludge (Barritt, 1940). No transitions were noted in samples taken at various intervals along the channel system.

The extent to which the fauna is influenced by the purification process was tested by treating crude sewage from Knostrop sedimentation tanks in an activated sludge system in the laboratory. Variations in the fauna, resembling those in a synthetic sewage sludge (Barritt, 1940), were observed as the sludge developed (Barker, 1943*b*). Initially the sewage contained few Protozoa, ciliates being generally absent, and at this stage clarification took over 24 hr. to accomplish. After 10 days' aeration and agitation, during which time sewage replacements were made and the settled sludge retained each time, the fauna became luxuriant, embracing a large abundance and variety of Protozoa from the Rhizopoda, Mastigophora and Ciliophora. New volumes of sewage were clarified after 4 hr. aeration and this stage displayed similar faunal characteristics to Barritt's (1940) 7-day sludge which showed poor 'settle-ability'. Finally, a restricted fauna, composed chiefly of Peritricha and *Paramoecium* in moderate abundance, was established after one month of aeration and new volumes of sewage were clarified even more rapidly (under 3 hr.). This condition, resulting from sewage with an original Biochemical Oxygen Demand of approximately 20, would appear to be equivalent to the 'climax condition' of Barritt, in which the original liquor had an approximate B.O.D. of 55. Barritt noted that this latter sludge was more

TABLE 2. *Showing the analysis of insect grazing organisms, total insect grazing organisms per sq.ft. and total ciliates per sq.cm. at Knostrop*

Fortnight ending	*Metriocnemus*	*Spaniotoma*	*Psychoda alternata*	*Psychoda severini*	Total larvae	Total ciliates
25 Jan.	17	12	0	0	29	—
8 Feb.	3	2	0	1	6	1764
22 Feb.	10	27	0	1	38	156
14 Mar.	172	44	0	745	961	227
28 Mar.	14	18	1	59	92	1772
11 Apr.	5	17	0	46	68	1763
25 Apr.	86	3	1	3683	3773	1145
9 May	490	74	7	242	813	611
23 May	137	113	135	232	617	651
6 June	130	329	455	85	999	652
20 June	16	347	165	23	551	2008
4 July	2	340	33	5	380	988
18 July	66	627	81	15	789	734
1 Aug.	94	1800	167	15	2076	1031
15 Aug.	103	3920	163	9	4195	601
29 Aug.	46	1994	48	6	2094	1041
12 Sept.	55	4120	59	0	4229	740
26 Sept.	55	2300	9	4	2368	1164
10 Oct.	144	3265	1	4	3414	968
24 Oct.	86	4080	32	20	4218	590
7 Nov.	40	2040	3	4	2087	2704
21 Nov.	214	2340	0	0	2554	313
5 Dec.	191	2341	0	43	2575	1826
19 Dec.	226	830	0	5	1061	729
2 Jan.	296	1310	0	3	1609	3837
16 Jan.	18	796	0	5	819	1877
30 Jan.	2	5	0	1	8	520

efficient than the 7-day sludge both as regards purification and 'settle-ability'. Thus clarification rate varied in association with changing protozoan populations, and tests were therefore made to discover whether clarification or flocculation depended on the presence or absence of a protozoan fauna. Two flasks of sterile wheat medium (A & B) were inoculated with a mixed culture of bacteria obtained from sewage and were incubated at 22° C. for 7 days. Flask B was then inoculated with a sewage culture of Protozoa, containing chiefly *Paramoecium*, and the two flasks again incubated for 7 days, the second flask A acting as a control. Before the inoculation of Protozoa into flask B, both flasks had been turbid due to bacteria in suspension, but after the second 7-day period flask B showed a flocculent mass of bacteria at the bottom with a clear supernatant fluid above, whilst flask A remained turbid with no flocculation. Since new bacterial types might have been introduced with the protozoan inoculum, the test was repeated using as the inoculum two *P. caudatum* individuals, freed from bacteria by washing (Hargitt & Fray, 1917). The organisms colonizing the flasks thus remained the same throughout the experiment with the sole exception of the addition of *Paramoecium*. The results confirmed those of the previous tests, flocculation occurring only in the flasks to which the pure *Paramoecium* had been added.

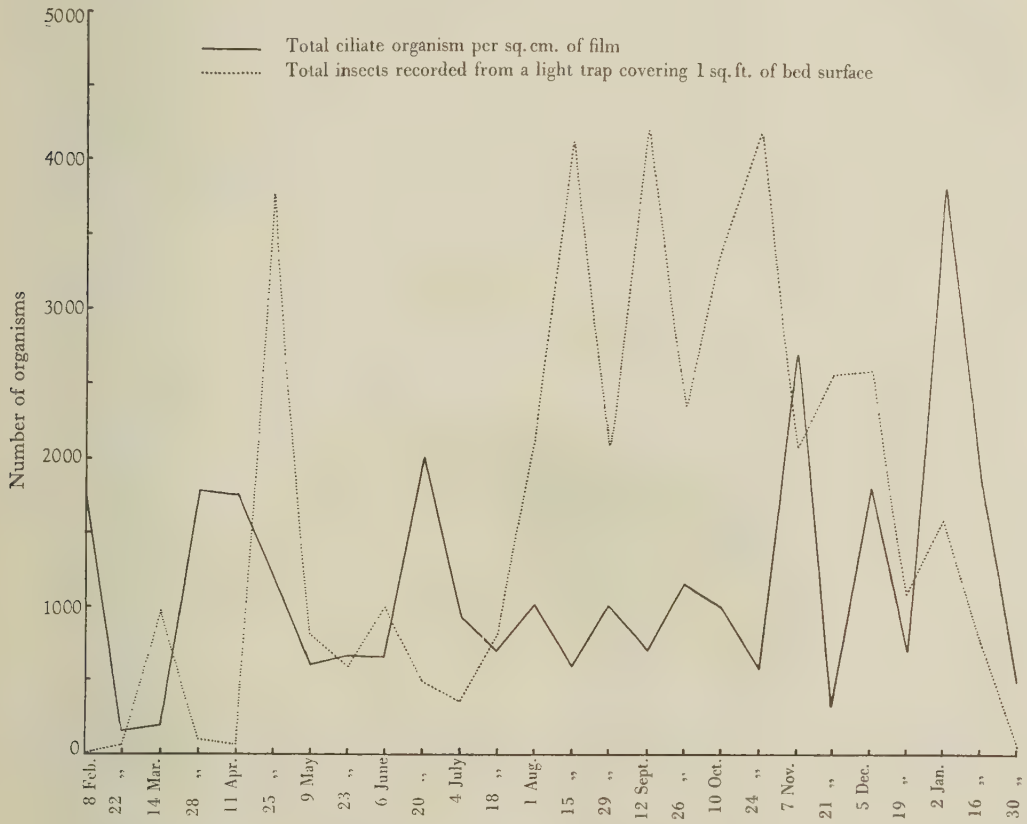


Fig. 3. Showing the relationship between the total insect grazing organisms and the total ciliate organisms in the bacteria beds at Knostrop.

Discussion

Investigations of the Protozoa occurring at several sewage works in the West Riding of Yorkshire have demonstrated a complex of species common to all (p. 315) with other less important forms occurring in the individual localities (p. 316), the presence of the latter being attributed to variations in drainage or in sewage conditions. The fauna of the sedimentation tanks (Barker, 1943*b*) is characterized by sparse pollutional forms, whilst that of the humus tanks is

much more varied, the pollutional types, several of which may survive the rapid passage through the system, being able to develop to some extent near the accumulating humus. The most striking changes occur in the bacteria beds where a grading of forms was observed as noted by previous workers (Johnson, 1914; Lackey, 1924, 1925; Cutler *et al.*, 1932). Thus, *Arcella* first appears a few inches below the surface of the Knostrop and Oulton beds, and other forms (e.g. *Spirostomum* and *Stentor*), though not present near the top of the bed, undoubtedly exist in the lower regions since they frequently appear in the bed effluent. At Bradford, where the sewage is stronger, Amoebina and flagellates were most abundant near the surface of the bed, ciliates near the centre and Conchulina near the base. These changes no doubt reflect a grading in the bacterial population which may be logically assumed to occur in vertical steps or zones in the filter (Holtje, 1943). In the movement of the sewage through the plant there is not time or opportunity for a climax holophytic association to develop fully, though a few holophytic types have been recorded in the humus tanks. However, Lackey (1938) has followed these transitions farther and has demonstrated that, in the

TABLE 3. *The distribution of Protozoa in depth in the Bradford bed: average numbers per sq.cm.*

Organisms	Depth in ft.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Arcella	1	1	1	1	—	—	—	—	—	—	—
	3	9	9	4	—	—	—	—	—	—	—
	5	1	36	18	—	—		—	—	—	—
Trinema	1	—	—	—	—	1	1	1	4	1	1
	3	—	—	—	—	4	1	4	1	1	1
	5	—	—	—	—	1	4	40	14	6	4
Amoebina	1	27	18	9	9	9	9	23	36	19	456
	3	145	34	4	1	340	9	1	9	23	134
	5	27	14	1	4	9	32	4	49	9	61
Flagellates	1	227	420	34	260	250	126	158	797	572	828
	3	804	125	68	90	214	92	125	170	260	158
	5	885	162	148	69	82	137	125	329	72	80
Chilodon	1	1	4	32	68	—	4	9	23	45	1
	3	1	36	63	18	—	32	207	122	166	23
	5	9	114	27	1	—	14	4	32	57	9
Cyclidium	1	—	14	—	—	—	1	1	14	3	—
	3	—	9	—	—	—	1	4	14	6	—
	5	—	4	—	—	—	4	1	1	3	—
Cinetochilum	1	9	1	1	—	—	1	—	—	1	1
	3	1	4	18	—	—	1	—	—	3	4
	5	9	14	18	—	—	14	—	—	1	1
Carchesium	1	—	32	91	1	—	1	—	—	6	4
	3	—	27	27	4	—	1	—	—	3	1
	5	—	72	268	1	—	4	—	—	6	1
Opercularia	1	1	100	68	1	32	1	4	23	15	18
	3	9	317	54	54	68	4	27	13	24	4
	5	1	337	18	23	4	1	1	4	3	1
Podophrya	1	1	1	18	—	—	1	1	—	1	—
	3	45	1	32	—	—	4	4	—	3	—
	5	9	45	132	—	—	1	4	—	6	—
Other ciliates	1	1	109	14	—	1	9	9	4	18	14
	3	1	136	45	—	9	1	1	31	14	4
	5	9	118	114	—	4	36	1	14	9	1

TABLE 4. *The relationship between the abundance of Protozoa in the beds and in the humus tanks of three localities*

Bacteria beds

Mean abundance of Protozoa per sq.cm.

Locality	*Sarcodina*	*Mastigophora*	*Ciliophora*
Oulton	2599	7770	1740
Knostrop	2242	3095	1170
Bradford	1242	1989	781

Humus tanks

Mean abundance of Protozoa per ml.

Locality	*Sarcodina*	*Mastigophora*	*Ciliophora*
Oulton	45	381	20
Knostrop	89	658	48
Bradford*	100	801	55

* Bed effluent reading taken in place of reading from the humus tanks.

River Scotia, U.S.A., the more heterotrophic fauna near the zone of pollution by sewage effluent is gradually superseded by an association in which holophytic forms predominate.

Certain specific seasonal preferences have been noted (Barker, 1943*b*): *Opercularia* shows positive correlation with temperature in winter at Knostrop though during summer its incidence is dominated by other factors. It is also probable that low temperature accounts for the absence of other forms in winter, e.g. *Euglypha* and *Stentor*, but does not explain the winter maxima of certain other species, a possible exception being *Chilodon* sp. which in both laboratory and beds thrives best at rather low temperatures. (In the laboratory this form was significantly more abundant in sewage cultures maintained at 5° C. than in cultures at 15° C. and in the Knostrop beds an inverse correlation with temperature was noted—Barker, 1942*b*.) Depression of other species in summer (p. 316) may result from the increased scouring activities of insects acting on an otherwise more or less uniform population. The general trends in the protozoan populations, however, can best be explained in relation to conditions in the beds and by considering the better known requirements of the Protozoa in the activated sludge system.

In activated sludge, conditions differ from those of the bacteria beds in that the medium or substratum, upon which the purifying organisms depend for their retention in the flowing sewage, is continually carried through the system and returned, after settling out in the humus tank, with the initial sewage entering the channel system. Consequently the protozoan fauna is uniform from the beginning to the end of the channel system, but local conditions (e.g. sewage strength, degree of aeration or time in the channels) may stabilize the fauna at a certain stage of the natural transition comparable with some stage in the filter process.

Barritt (1940), confirming observations by Lockett (1928) that flagellates were numerous in activated sludge only when the effluent was of poor quality and that ciliates predominated when the effluent was good, also showed that sludge in the latter case was more efficient in purification of the sewage so that, once established, it modified the environment in favour of the conditions in which it originally arose. This process is probably associated with the bacterial-protozoan interrelationships and would not always work in favour of the protozoan fauna as, when agglutination is exceptionally efficient, the bacteria may be made so completely unavailable as food for the Protozoa that the latter are depleted or at any rate reduced (Barritt, 1940). The early stages in this restriction are believed to be present when only those strong swimming and sessile ciliates, capable of searching large volumes of water for food, are present (e.g. *Paramoecium* and *Vorticella*). In this respect it is interesting to note that of the bacteria beds investigated those of Oulton show the greatest abundance of Protozoa (considering samples only from the upper region, as comparable samples were not available from greater depths) but that the corresponding humus tank populations are the most scanty. This feature may indicate a depletion of food by direct removal or by agglutination corresponding to that noted above in an efficient activated sludge. Fluctuations in the humus tanks at Knostrop follow those of the beds (Barker, 1942*a*) and the population is varied, though an abundance of Peritricha indicates an association more closely related to that of a less efficient activated sludge producing a medium degree of purification.

An analogy again exists between the beds and the activated sludge system, for at Bradford, with its 'strong' acid sewage, the upper regions at least tend to a pollutional association with flagellates predominant. Ciliates, though more abundant in the middle depth than at the surface, are usually scanty. With the onset of sloughing, however, the more open beds give rise to a better effluent with some nitrate present and the Protozoa exhibit a tendency to a higher grade in transition with general increase in ciliates and rhizopods and some decrease in flagellates. At Oulton, however, where the sewage is weak and the beds always open, a very high grade in the transition is normal with flagellates relatively scanty. Sloughing in this latter environment is accompanied by a general reduction in the protozoan fauna suggesting that conditions are carried beyond the optimum and a state of depletion is reached equivalent to that which Barritt (1940) envisages for the very old efficient sludge.

At Knostrop, with its medium 'strength' sewage and all groups of Protozoa well represented, sloughing had no limiting influence on the seasonal trend of the Protozoa. Here, the intensive grazing of insects, the populations of which are more abundant than at Oulton or Bradford (Lloyd, verbal communication), appears to be chiefly responsible for the fluctuations in the protozoan populations. The indirect relationship between insect activity and protozoan abundance (p. 318) is no doubt operative all the year round, but in winter larval activity is reduced by the low temperatures (Lloyd, 1943). Further, at this time, there is a prolonged interval between the larval stage and the emergence of flies so that the latter cannot be taken as a proximate index of activity. Thus, during summer, when peaks of larval activity occur within a few days of the peaks of fly output, correlation between the latter and protozoan abundance is good, but during the winter any relationship would be masked by a time lag. This observation is important since it explains in general terms the reason for the fluctuations of the ciliates and also indicates the thoroughness with which the grazing larvae work

over the film. Finally, it shows that the method of sampling, by means of slide film suspensions, gives a fair assessment of the Protozoa in the beds since their numbers show definite correlation with those of another class of animals reached by quite different sampling methods.

Limitations of the Protozoa in the bacteria beds may therefore result from different environmental conditions, but at Oulton the population of the beds became so dense during January that limitations of certain species could possibly result from overcrowding. At this time, allowing for considerable undulations in the film, $\frac{1}{18}$ of the total area would be covered by ciliates, of which about 98% were active free-living forms and chiefly *Chilodon* sp., $\frac{1}{720}$ by flagellates and $\frac{1}{3000}$ by rhizopods, so that the whole of the surface area would be traversed every few seconds. This crowding, together with the considerable activity of the free-living forms, would no doubt exert an appreciable effect upon the settling of organisms such as would be necessary for the establishment of the Peritricha and certain flagellates in the film. A form of competition would then be at work in which space and not food would be the controlling factor.

The agglutination of bacteria into flocs has been recorded and referred to by several authors. Lockett (1928) showed that purification in the absence of Protozoa resulted in an effluent which was turbid when compared with one in which Protozoa were active during purification. Viehl (1937) also noted that Protozoa were effective in producing flocculation in activated sludge, while Barritt (1940) showed that this process was most efficient in an old sludge supporting a protozoan population restricted almost entirely to a few species of ciliates. In the laboratory tests, an increase in the protozoan population increased the rate of clarification of new additions of sewage, a feature which was most pronounced when ciliates became predominant. In flask cultures clarification was not observed inside three weeks unless Protozoa, free from or contaminated with bacteria, were added to the flasks in which case rapid agglutination of the bacteria resulted. In the present work it has been demonstrated that *Paramoecium caudatum* is capable of bringing about this agglutination, while Pillai & Subrahmanyan (1942) have stated that both *Epistylis* and *Vorticella* are also effective, the former being more efficient than the latter. Hardin (1943) and Watson (1943) have demonstrated the ability of other protozoan species, *Oikomonas termo* and *Balantiophorus minutus* respectively, to bring about agglutination, the former using pure cultures of different species of bacteria and bacteria-free Protozoa. On the other hand, Jenkins (1942), who suggests that 40% of total purification of sewage may result from flocculation, maintains that the process is not dependent on the presence of Protozoa and in this respect supports Butterfield & Wattie (1941). Recently, however, Watson (1945) has demonstrated a mechanism by which the agglutination is effected by the ciliate *Balantiophorus minutus*. It would seem therefore that, in mixed cultures of bacteria, as in sewage, the liquor remains turbid due to bacteria in suspension and that flocculation is greatly enhanced if not brought about completely by the activities of the Protozoa.

The observations of Lockett (1928), which indicate more complete chemical breakdown in the presence of Protozoa, and Viehl (1937), that Protozoa are essential for the complete breakdown of complex proteins, indicate a further role for these organisms in sewage purification. Barritt (1940) suggests that the organisms surviving in efficient activated sludge may live by the direct removal of organic matter from solution, the bacteria being unavailable as food, and Reynoldson (1942) has shown that there is a direct relation between the degree of purification and the number of vorticellids present. Explanation of the increased purification in the presence of Protozoa may be found in the supposed saprophytic mode of nutrition of the ciliates, a view supported by Pillai & Subrahmanyan (1944) who claim that *Epistylis* alone, in aerated faecal emulsion, is capable of effecting purification to such a degree that the bacteria are regarded as of secondary importance in the process. Alternatively the increased purification could result from an increase in the rate of the physiological activities of the bacteria due to the predatory activities of the Protozoa (Cutler & Bal, 1926; Meiklejohn, 1932; Harvey & Greaves, 1941). In working plants, such effects may accompany large variations in the protozoan populations, but at Knostrop, where chemical and biological analyses were carried out on the same samples, no sign of increased purification corresponding to increase in Protozoa was noted. At Bradford, however, increase of purification during the slough, evidence of which was found in the increased quantities of free oxygen, ammoniacal nitrogen, nitrites and nitrates, corresponded to a marked increase in the ciliate and rhizopod populations in the beds, but whether the increased purification was due in any measure to the increased population is obscure.

An adequate supply of oxygen during purification is essential for the production of the desirable end products, and in this respect Lloyd (1945) indicates a further role for the protozoan populations of the bacteria beds, namely, that the flagella and cilia of the organisms not only bring them food, but also serve to aerate the water, an effect which, though not considerable in bulk sewage, must be potent in drops and films inhabited by such considerable numbers of Protozoa. Finally, the Protozoa form a vital link in the food chain and faunal pyramid of numbers within the bed, Protozoa preying upon bacteria and

themselves being preyed upon by insects, etc. (p. 318, and Pillai & Subrahmanyan, 1942). In this way the substances in solution and in suspension are removed and retained to some degree in the bodies of the smaller organisms which remain suspended in the sewage, these organisms being removed in turn by larger organisms which escape as flies or settle out in the humus tanks, thus helping considerably in the purification of the sewage.

My thanks are due to the following for permission to visit their respective works and for supplying data relating to the plants: Mr J. T. Thompson, late of the Leeds Corporation Sewage Works at Knostrop; Mr H. Wontnor Smith, sewage engineer, and Mr Beedham, chemist, of the Bradford Corporation Sewage Works; Mr G. Wilkinson of the Oulton Sewage Works; Dr H. H. Goldthorpe of Huddersfield Corporation Sewage Works, and Mr G. H. Fretwell of the Rodley Sewage Works. I also wish to express my sincere thanks to Dr Ll. Lloyd for invaluable advice and discussion and for supplying counts of the insect populations. Finally I should like to thank Prof. E. A. Spaul for his interest throughout the course of the work and for his help in the revision of the manuscript.

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Proceedings of the Association of Applied Biologists

Joint Meeting of the Association of Applied Biologists and of the Microbiological Panel of the Food Group of the Society of Chemical Industry held on Friday, 12 April 1946, in the London School of Hygiene and Tropical Medicine. Chairman at the morning session: Prof. W. Brown, Vice-President of the Association. Chairman at the afternoon session: Prof. B. T. P. Barker, Chairman of the Microbiological Panel.

Symposium on problems associated with the growing and treatment of flax

Morning session:

Research and the flax industry. By G. O. Searle.

The diseases of flax, with special references to seed-borne diseases and their control. By A. E. Muskett and J. Colhoun.

Afternoon session:

Recent developments in the retting of flax and the dispersal of waste water. By T. W. Brandon and C. B. Taylor.

Recent developments in the microbiology of retting. By L. A. Allen.

(*Note.* The papers given in the afternoon session are reported in *Chem. & Ind.* for 25 May 1946, p. 205.)

Research in the flax industry

By G. O. Searle, *H.M. Norfolk Flax Establishment*

The flax industry includes all the operations from the sowing of the flax crop to the despatch to the spinners of the fibre which is their raw material for linen manufacture. The scope for scientific investigation is wide as it has to cover not only the many botanical and agricultural problems involved in growing the best crop of flax, but also the physical, chemical and engineering problems of obtaining the best spinnable fibre from it.

For those who are not very conversant with the flax industry a few introductory remarks in explanation may be of help.

The industry is very old, and in essence the production of fibre for spinning has always followed a simple sequence which up to quite recent years was more a handicraft than a mechanized industry. The crop is grown much like any other white straw crop and is harvested by pulling instead of cutting. The seed is removed from the crop, although this is not essential and in fact is not done in the rural methods of flax production in Northern Ireland, and the straw is soaked in water, retted as we call it, dried and then beaten to remove the woody parts of the stems from the long fibre strands. That is the sum of the traditional operations involved in flax production, and superficially looks simple. Indeed in the days when flax production was entirely a rural industry and labour was still cheap there can have been very little call for scientific investigation, and the accumulated experience of hundreds of generations of flax craftsmen with their simple implements was sufficient to provide all the types of fibre required by the spinners.

In fact one must confess that the flax workers could produce finer flaxes, and hand spinners could spin finer yarns 100 years ago, or 1000 years ago, or indeed I might truthfully suggest 5000 years ago, than they can at the present day with all the blessings of science to help them. Perhaps that thought introduces the right note of humility into this endeavour to outline to two scientific societies some of the problems of research in the flax industry.

In this connexion it may be interesting, and not totally irrelevant, to relate the duly authenticated fact that in the years 1800–15 an old woman of County Down, Anne McQuillan by name, quite often prepared and spun flax as fine as 60 or even 80 hanks to the lb. and in one case reached 105½ hanks to the lb. Now as a hank contains 12 cuts, and a cut consists of 120 threads and each thread is

$2\frac{1}{2}$ yards a little mental arithmetic will, I hope, give you the same result as I got, namely, that this dear old lady at her best was producing by hand a yarn of the surpassing fineness of 215 miles to the lb. weight. I think she could almost claim to have antedated nuclear fission by 130 years.

However, we are now in an industrialized and mechanical age. Mass production has to take the place of handicraftsmanship, and science has to provide the wherewithal.

Although the range of flax problems is very great a definite unity of purpose runs through them all. Broadly speaking, our main purpose is to produce the greatest possible yield of spinnable fibre per acre and to conserve the maximum proportion of this fibre from the straw stage until its conversion into yarn. Admittedly the flax industry proper ends at the sale of scutched flax, but as our end product is the spinners' raw material we must plan our methods to suit their utilization of our product.

With this in mind I suggest we can conveniently divide the problems of the flax industry to-day into seven sections as follows:

(1) *The botanical section.* Producing types of flax potentially capable of growing crops to best advantage under various local conditions in this country, and potentially suitable for processing to best advantage in the flax mill, e.g. high fibre yielding types, high spinning quality types, weather resisting types, disease resisting types, and possibly 'easy retting' types, and 'easy scutching' types if these characteristics are found to be heritable.

(2) *The agricultural section.* Discovering the environmental conditions under which such types may be grown to best advantage, e.g. their reaction to various soil types and agricultural operations, to times, rates and methods of sowing, to climatic conditions, to manuring both with major and minor elements, to weedicides and fungicides, and to hormones.

(3) *The harvesting section.* The mechanical and other problems involved in harvesting the crop in optimum condition and transferring it to the flax mill in optimum condition.

(4) *The storing section.* Determining the optimum conditions for maturing, conditioning and storing flax crop, de-seeded straw and retted straw so that they may be presented to mechanical processing to best advantage.

(5) *The retting section.* Determining the straw treatment necessary to ensure that the subsequent mechanical treatment gives the highest yield and spinning quality of long fibre consistent with that inherent in the type of straw being processed, and at the most economic cost.

(6) *The fibre separation section.* Solving the mechanical problem of long fibre separation without waste, both with retted and unretted straw.

(7) *The by-products section.* The economic utilization of all products other than scutched flax, i.e. the seed, the chaff, the cavings, the weed seeds, the tow straw, the scutching rug, the tow, the shives, the dust, and, if possible, the retting effluent.

All of the problems I have mentioned are applicable to a modern flax industry on a factory basis. Many of them are equally applicable to the rural flax industry which still, in various countries of the world, is the principal source of flax for the linen industry.

In order to get the picture in proper perspective it may be of interest for a moment to consider the potentialities of flax research which would seem to be extensive, perhaps owing to the late date at which flax production started to become industrialized. Flax crop is the primary product, scutched flax, i.e. the long fibre strands free of extraneous wood, is the intermediate product and the unboiled flax yarn is the final product so far as the production and conservation of fibre is concerned.

In exceptional cases we have grown flax crops of 4 tons dry weight to the acre. A 3 tons per acre average should be quite within the bounds of possibility. Studies we have carried out of the total fibre content of flax straw show that any reasonably well grown straw contains 20% of fibre in the long strand form. From a pedigree flax properly grown 25% should be obtainable. We thus have a potential 15 cwt., or as usually reckoned 120 stones, yield of scutched flax per acre. During the war years the average yield in these islands was of the order of 24 stones. Before the war 32 stones was a fair figure. In Belgium with their superior handling they were averaging 40 stones or more, but in Russia only about 12 stones. At a conservative reckoning, therefore, we have a 200% increase to look for.

When the scutched flax reaches the spinning mill it is first hackled. Hackling is partly to clean the fibre from particles of wood, epidermal material and cortical tissue but mainly to split the fibre strands down to finer strands ready for the preparing systems prior to spinning. A 60% yield of hackled line from scutched flax is fairly good. Of the 40% combed out some 5% is waste and the remainder is made up of fibre strands which have been reduced to the status of tow. During the remaining processes up to the production of yarn very little fibre is lost although there is a certain amount of extraneous material removed. Roughly speaking, therefore, and allowing for non-fibrous waste, 50% of the scutched flax reaches the line yarn stage in normal practice.

I think it quite possible, however, that one might combine the scutching and hackling operations and in fact we have already made some small progress towards this. In such a case one might hope for possibly an 85% yield of yarn from scutched flax or, converted back to crop, a potential yield of over

12½ cwt. of yarn per acre against the present average of about 2 cwt.

When in addition I tell you that, leaving yield of fibre on one side, there was before the war a differential in value of scutched flax according to its spinning quality of over £100 per ton, I think you will agree that a little scientific investigation into the why and wherefore of flax production should hardly prove to be the thankless task of gilding a lily.

Starting with the first of my seven divisions of flax research, the botanical section, I can report that fair progress has been made. In fact, one of my colleagues has been continuously concerned with the flax breeding problem for 26 years. The earliest attempts at improving flax yields by single plant selections were only made about 1909, and concerned themselves in the first instance with the production of taller strains to give a higher tonnage per acre. One of these strains, the 'J.W.S.' flax originated by Dr J. V. Eyre, eventually reached commercial bulk and was widely sown in Northern Ireland and Canada. The production of new lines with the specific object of increasing the percentage of fibre in each straw started in 1920 at the Linen Industry Research Institute in Northern Ireland and was transferred complete to Norfolk in 1935 and is still being carried on there by my colleagues. The basis of this work is the percentage of fibre in the single stem as determined by measurement of cross-sectional areas at a pre-determined point, namely, half way between the cotyledon scar and the base of the first flowering branch, and at a constant date in the plants maturation period, namely the 35th day after the first flower opens. Preliminary studies in 1920 and 1921 had shown that this factor, percentage of fibre, was strongly inherited, as also was the relative height of the mature plant. A large number of other characters have been measured during the long course of the investigation, but percentage of fibre and height are the ones of most practical importance so far. The initiation of this study involved the working out of a new technique for the mass production of transverse sections, as it was necessary to have permanent mounts of sections from several thousand stems made each winter. Briefly the method involves the celloidin imbedding of short pieces of stem which are then microtomed at about 18 μ. After double staining and clearing three sections of each stem, as perfect as possible, are mounted in canada balsam. The measurement of stem area and fibre area is done by a planimeter on a projected image at a suitable magnification. During the course of the work approximately 60,000 stems have been examined by this method.

The new strains produced in this way were bulked up and placed on the market under the name of Liral varieties, and during the late war the Liral Prince, Liral Monarch and Liral Crown, together with the equally good variety Stormont Gossamer propagated by the Ministry of Agriculture, Northern Ireland, have reached the very satisfactory status of being the sole varieties used for sowing the enormously expanded war acreage in Great Britain, Northern Ireland and Eire, together with the greater proportion of the flax acreage in Canada, Australia, New Zealand and Kenya. In 1945 and 1946 for the first time in history we have been in the happy position of exporting considerable quantities of flax sowing seed to the Continent instead of importing all our requirements from the Continent as was invariable up to ten or fifteen years ago.

The actual improvement achieved so far is shown by the results of Ministry of Agriculture trials in Northern Ireland over the past fifteen years where the four varieties mentioned have invariably shown an increased scutched fibre yield of 50% or more per acre compared with the commercial varieties which used to be imported from the Continent.

Whilst still further improvement in yield is being sought and successors to the Liral varieties may soon begin to appear under the label of Norfolk varieties, the main emphasis of this work is changing to the study of fibre quality and other important factors. Spinning quality is a far more difficult characteristic to study than fibre yield largely because at the moment it cannot be tied up with any easily recognizable character in the single straw. It is, therefore, necessary at present to carry potentially hopeful strains to the stage when they will produce sufficient fibre for a spinning test, which enormously lengthens and complicates the early stages of flax breeding. One ray of hope lies in the discovery of a distinct indication that a linkage exists between spinning quality and depth of flower colour, although I certainly should not like to be asked to define in chemical or physical terms how such a connexion could be explained. At various times we have identified thirteen different gradations in tone between the pure white flower and the dark violet flower and their relation to spinning quality is a line of work well worth following up.

I will not say anything about disease resisting types as this paper is to be followed by one from Dr Muskett the acknowledged authority on flax diseases. All I will say is that we have so far been able to pass on one strain, the Norfolk Queen, which we understand shows some merit in wilt resistance. We gather that a good deal of work on rust resistance in flax is in progress in Australia, and we have also received some varieties said to be rust resistant from the U.S.S.R. Owing, however, to the various physiological races of *Melampsora lini* I fear that the breeding of rust resistant races will be a very knotty problem.

In the botanical section also there is certainly a lot of physiological and morphological work still to be

done if we are properly to understand the structure and development of the fibre network which is the basis of our flax processing. We did carry out, some years ago now, a fairly comprehensive study of how the fibre strands run in a normal flax stem. A method was devised for stripping the whole fibre system from a single stem without deranging the strands or the ultimate fibres and of mounting it complete on a long glass slide. We were then able to cover the whole fibre system by 700 $\frac{1}{4}$-plate photographs in polarized light. These were built up into a composite photograph approximately 75 ft. long which showed very clearly the course of every fibre strand from one end of the stem to the other. Amongst other things it demonstrated for the first time the real reason why the hackling of scutched flax always produces more root end tow than top end tow. Hackling is carried out by combing from the middle of the piece of scutched flax towards the root, and then from the middle towards the top. In the stem itself the strands composing the fibre system all originate at the root end and run to the top end, but at each leaf trace a whole fibre bundle runs out, its place being taken by the forking of another fibre bundle higher up. It was thus clear that in hackling to the root end the pins could catch in these bundle endings and tear out a large portion of a bundle, but in hackling to the top end there was no such obstruction to the passage of the pins.

In my second division which I have called the agricultural section there are obviously a number of problems which are common to all types of crops as well as flax. For many years flax manuring trials have been carried out by the Ministry of Agriculture in Northern Ireland, and based on these experiments the tradition quite rightly grew up that potash was the main requirement for a good flax crop. During the past four or five years very extensive flax manuring trials have been carried out in this country by the Ministry of Agriculture, the produce being processed at the Norfolk Flax Establishment and the results being studied at Rothamsted. These trials have established without doubt that in this country flax shows no response to potash, or for that matter to phosphates, unless the soil is classed as markedly deficient in those elements. The explanation of these contradictory results is of course that generally speaking the soils in this country, at least in the principal flax-growing areas, are much less deficient in potash than those in Northern Ireland.

The general problem of the effect of the environmental conditions on the growth, processing and products of the flax crop is at present being attacked by means of two statistical surveys of the principal factors involved. One survey covering a number of crops in a flax area in County Tyrone is being carried out by the Linen Industry Research Association, and the other covering a very large number of crops from every flax-growing area in this country is being carried out by the Norfolk Establishment. The results of both these surveys promise to be of exceptional practical interest, but neither have been completed as yet.

Dates and rates of sowing and spacings of drills are problems of special interest in flax. Theoretically, as flax does not, or rather should not, tiller, uniform distribution of the seed, i.e. the narrowest drilling possible, should give the best crop. We have worked out that one plant to every $1\frac{1}{4}$ sq.in. is the ideal. We are now beginning to haver, however, and think it is possible that we do not know as much about it as we thought we did twenty years ago. Perhaps this is a salutary state of mind to be in. There is no doubt, however, that the length of the growing period, and more particularly of the period from first flowering to harvest is a very interesting study in flax and of the greatest practical importance. The development of the fibre strands lags behind the development of the stem in length. One wants to harvest flax at the point when the fibre has reached its full development and yet lignification has not set in, and also at the moment when the seed is sufficiently developed to complete its maturation in stook. In this country, where we may have very hot harvest weather and very rapid changes in the straw, it is not easy to hit off this optimum moment exactly. Some studies of the maturation period that we are making now seem to show the interesting fact that whereas at flowering the moisture content of the base, middle and top of the stem and the seed and the seed capsule are very far apart, as maturation proceeds all the curves converge until at about the 30th day after the appearance of full flower they cross at one point and then diverge again. There are indications that this date at which the moisture contents of the several parts of the flax straw are uniform is the right moment for harvesting.

The effect of herbicides on flax, particularly the newer D.N.O.C. compounds, is the special province of Prof. Blackman, and we have done nothing but process a number of his plots for him. I think he would agree that so far as flax is concerned there is a lot of work still to be done, and it is more difficult than with the cereals. One must take into account the effect on yield of fibre as well as on the yield of straw per acre. Progress, however, is definitely promising.

On the subject of fungicides I will leave the field clear for the paper which follows mine, though we have a fairly extensive programme on the subject in Norfolk.

Most of the remaining sections of flax research get perhaps rather away from the direct interests of either the Association of Applied Biologists or the Society of Chemical Industry, in fact the interest is largely engineering. I will, therefore, try to be brief with them.

The harvest machinery section is of some interest. Flax is pulled and not cut. And for thousands of years it was always hand pulled which was a laborious job. The first crude pulling machines were tried in 1919 and 1920, but little more was heard of machine pulling until I tried the first really practical pulling machine, one of Messrs Boby's, in 1933 in Norfolk. The start of the war and the enormous expansion of flax growing in this country forced us into a very large pulling machine programme, and I can assure you that in the early years some of the teething troubles nearly sent us crazy. We are not in the fortunate position of Northern Ireland of having a long tradition of hand pulling and a fair supply of hand pullers available, and I always had before me the bogey of the 1918–19 flax expansion where accounts showed that flax pulling in England with untrained boys and girls had sometimes cost £50 per acre. Too much praise cannot be given to Messrs Boby who made magnificent efforts to supply all the machines required and constantly to improve them each year. I believe that the latest model—the Boby-Vessot—will come as near to being the perfect flax harvesting machine as we shall see for a number of years.

The straw has to be gripped between rubber belts, tweaked out of the ground, carried up to and across a transverse platform and delivered into a binder head. The main desiderata are a reasonable daily performance, say 8–10 acres a day; the keeping of the root ends level hence the necessity of pulling in narrow swathes; maintenance of the parallelism of the straws; freedom from damage from crushing or nipping and the eventual delivery of beets of flax quite separate from each other.

The proper handling of the straw during harvesting is quite half the battle of flax processing. Mishandling at harvest entails loss and waste of material at every later stage.

My fourth section concerns the storage of flax both in the crop, de-seeded straw and retted straw forms. It sounds rather dull and simple, but we believe it may be important. There are undoubtedly certain somewhat obscure physical and chemical changes which take place during storage, outside the more obvious things like mould growth if the straw is too damp. The skilled Belgian flax workers undoubtedly know of these changes empirically and take account of them in their handling of flax. They lump them together under the general term 'conditioning'. So far we have followed up the changes in moisture content pretty thoroughly particularly of straw in stack, and it is surprising how quickly conditions in the centre of a 25 ton stack can change in conformity with changes in the outside conditions, it is only a matter of seconds. On the chemical side I confess we know practically nothing, though we have made a start with the pectin and cellulose chemistry it probably involves.

My fifth section concerns retting and is one of the most important and complex of all. During the war, as you are doubtless aware, we in this country concentrated almost exclusively on green (unretted) fibre production as it was found to give a material suitable for most war uses. Now in peace time we have to change over as quickly as possible to retted fibre as that is the main requirement of the spinner for the finer linens for domestic use. Whether or not, if the war had gone on a few years longer, the spinners would have advanced sufficiently in their technique of utilizing green fibre ultimately to prefer it to retted is a thorny problem I cannot enter into here. Perhaps you will agree that it was not worth the continuance of the war to prove such a recondite point.

You are having two papers this afternoon on certain aspects of retting, so I must not trespass on the ground they will cover at this point although it is a subject in which I am intensely interested and on which the Norfolk Establishment has a large programme in front of it. I think I can say without boasting that we are now completing in Norfolk the most exhaustively gadgetted experimental retting plant in the world and we hope to get down to some of the fundamental problems of retting which had to be by-passed during the war. Retting is a most interesting problem. In rural flax production, as exemplified by Northern Ireland before the war, retting is carried out by immersing the flax straw, seed and all in a dam cut in a field and filled with water. It turns out, in expert hands, a particular type of fibre which is highly esteemed. In Belgium before the war the best flax was steeped in crates in the River Lys and the resultant fibre was of a quality which we cannot touch at present.

To-day in flax mills in this country and in Belgium, flax is retted in concrete tanks. We can control the liquor ratio, we can control the temperature to a degree, we can change the water in any proportion or at any rate we like, we can aerate the water as you will hear this afternoon, and yet in my view we are no nearer having a controlled ret than we were a thousand years ago. We depend entirely on empirical tests for telling us the end point, and when we put the straw in the tank we should think ourselves very expert if we could tell within say 10 hr. when it should come out. The reason is that at present we are unable definitely to correlate any known chemical and physical properties of the straw with its retting behaviour and furthermore we are dealing with a retting liquor which is never of constant composition for two minutes on end. Those are the lines on which I feel future retting research must go, and it is a very pretty problem for a team of plant physiologists, biochemists, chemists, physicists, bacteriologists and enzyme experts, and last but not least the plant breeders because we did once breed and discard a

variety of flax which apparently rotted before it retted.

The fibre separation section of my suggested programme is purely engineering and I do not propose to waste your time with it now beyond saying that, having Government resources behind us, we were able in Norfolk to have designed and built a novel type of flax scutching machine embodying quite different principles to the ordinary turbine scutcher. With this machine we have some hopes that we may be able to combine the scutching and hackling operations, with a consequent improvement in the ultimate yield of yarn from flax straw. If any of you are up Norfolk way at any time and are interested in mechanics we shall be only too pleased to show you this and other items of mutual interest.

My last section concerns by-products. Under normal conditions from every ton of crop delivered one may hope to get 10% of sowing seed, 10% of scutched flax and 10% of tow. That leaves 70% to be accounted for. The major part is flax shives, or woody waste.

During the war one could sell almost anything at any price—as you probably know weed seeds were a very good trade. Still in peace time one must try to make the best use possible of by-products, if the flax industry is to be put on an economic basis. Any ideas on the possible use of flax by-products in peace time will, therefore, be very welcome. Many tons of flax shives, for instance, were sold for polishing tin plate. Thousands of tons of green flax rug have been sold as packing material for goods for the Far East. All our flax chaff has been sold as stock feed, particularly for sheep. Flax dust has gone as a filler for artificial manures.

I am still hoping that I can market the retting effluent in half-crown bottles for medicinal purposes. It is full of salts and tastes just as bad as the accredited spa waters.

Seedborne diseases of flax and their control

By A. E. Muskett and J. Colhoun

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During the last century or so the cultivation of linen flax has always been of greater importance in time of war than in time of peace. It was towards the end of the 1914–18 war that Pethybridge & Lafferty (1918) and Lafferty (1921) worked out the life-histories of *Colletotrichum linicola*, the organism responsible for seedling blight and *Polyspora Lini*, which is responsible for stem break and browning. These two seedborne parasites of flax are of major importance, and although their life-histories were well known by 1920 or thereabouts, no satisfactory method for their control other than the use of non-contaminated seed had been discovered.

Consequent upon the outbreak of occasional severe attacks of stem break and browning in the Northern Ireland flax crop in 1938, a method was devised whereby flax seed samples could be speedily and accurately examined for their contamination with *P. Lini* (Muskett & Malone, 1941). At the same time, it was found possible to apply the same method for the examination of seed for contamination with *Colletotrichum linicola*. The use of this, the Ulster method, enabled healthy samples of seed to be segregated from those contaminated with the parasites in question, and after obtaining bulk seed samples of heavily contaminated seed it was decided to investigate the possibility of controlling the diseases for which these organisms are responsible by the use of seed disinfectants. It was found that by slight adaptation the Ulster method could be used with reliability for the evaluation of seed disinfectants (Muskett & Colhoun, 1942), and as the method is applicable at any time during the year and the process of testing only occupies some 7 to 10 days, the tentative values of a large number of possible seed disinfectants could be assessed without recourse to field trials. As the result of laboratory investigation coupled with field experiments it was found that organo-mercurials, which have proved so satisfactory for cereal seed crops, were not so effective for flax. This is accounted for by the smoothness of flax seed with the consequent non-adherence of the dust. It was found that when organo-mercury dusts were used at the very heavy rate of 20 oz. per cwt. of seed, a promising measure of disease control resulted, but as such treatment was extravagant and involved the use of large quantities of a poisonous material, this method was impracticable. Increased weight was given to this decision on considering that almost all the flax acreage in Northern Ireland is sown by hand and therefore the sower would come very directly into contact with the disinfectant. At the same time, it was found that when an organo-mercury dust was adfixed to the seed by the use of water applied as for the short wet method of disinfection, very satisfactory results were obtained, but this method was

ruled out owing to its requiring a double disinfection process (Muskett & Colhoun, 1944, 1945*a*). The direct short wet method of disinfection (Muskett, 1944) using an organo-mercurial in soluble form was found effective, but this method could not be immediately operated on account of the lack of suitable machinery for its application to seed in bulk (Muskett & Colhoun, 1944). During the course of the investigation a number of products were submitted by the chemical industry for test purposes and one of these received from Messrs Imperial Chemical Industries Ltd. and labelled R.D. 7846 showed great promise. Used at the rate of 12 oz. per cwt. of seed it provided almost perfect control of *Colletotrichum linicola* and a high measure of control of *Polyspora Lini* (Muskett & Colhoun, 1943). This material contained tetramethylthiuramdisulphide as the active fungicide and was regarded as relatively non-poisonous. The findings in the laboratory were confirmed by a series of field experiments, and it was then decided to consider seriously the treatment of all flax seed with this disinfectant as a safeguard against the outbreak of epidemic disease in the crop through the use of contaminated seed.

Northern Ireland had undertaken to produce 100,000 acres of flax annually for the war period and the seed necessary for this crop had to be supplied from the United Kingdom crop. Flax was again to be grown under intensive cultivation from home-produced seed and the problem of the prevention of crop diseases caused by seedborne parasites had to be faced. When, through the good offices of the Ministry of Supply and Messrs Imperial Chemical Industries Ltd., it was found possible to have R.D. 7846 manufactured in bulk it was decided to undertake the disinfection of all flax seed produced in Northern Ireland. R.D. 7846 had been named Nomersan and arrangements were made to disinfect all seed produced for sowing purposes from the 1940 crop. Strickland seed dressing machines were installed at all the centres of production and the supplies of the necessary disinfectant were made available. In order to safeguard the farmer it was desirable to ensure the good quality of the seed before its disinfection and to attain this end all bulk samples of seed produced were examined for moisture content, purity and germination; those which did not reach the necessary standard required under the Flax Seed Order were rejected as unfit for use. All seed which reached the required standard was disinfected under expert supervision and the bags duly labelled and sealed. The operation worked smoothly in practice and through the willing co-operation of the processors it was possible to ensure that all sowing flax seed produced in Northern Ireland for the 1941 crop was of good quality and disinfected. Great Britain followed suit the following year and from 1941 onwards the disinfection of sowing flax seed had become universally adopted in the British Isles.

By 1942 *Botrytis* sp. the cause of Grey Mould had been detected as a seedborne parasite and disinfection was shown to be effective in controlling this disease as it occurred in the seedling phase of the crop (Colhoun, 1944). In the 1943 crop, however, a further difficulty arose owing to an epidemic outbreak of Foot Rot caused by *Phoma* sp. (Muskett & Colhoun, 1945*b*). *Phoma* had been detected in seed samples and had been found to be seedborne but as, until 1943, very little trouble had been caused by this parasite, it had received little or no attention. The seed from the 1943 crop was found to be heavily contaminated with *Phoma* and, at the same time, it was shown that seed disinfection with Nomersan was not nearly so effective for controlling foot rot as for other diseases. By using the Ulster method it was found possible to determine the extent of seed contamination, and in order to lessen as much as possible the risk of foot rot caused by seedborne *Phoma* in the 1944 crop, arrangements were made to withhold from sowing all seed showing contamination to the extent of more than 5%. It was hoped that by using slightly contaminated seed which would, in the normal course of events, be disinfected, the risk of a serious outbreak of foot rot in the 1944 crop would be avoided. A further difficulty arose in that the contamination of seed bulks with *Phoma* was found to be very uneven owing to the patchy nature of the occurrence of the disease in the crop. This meant that no method of seed examination could be regarded as reliable unless thorough mixing of the bulks of seed was achieved before tests were made. To overcome this, feeding-stuff mixing machines were purchased and installed at the centres of seed production so that seed bulks could be thoroughly mixed before examination. Tests showed that such mixing was effective and that the results from tests made with mixed samples could be regarded as reliable. At the same time, it was found that by incorporating the necessary quantity of disinfectant with the seed in the mixer the process of disinfection could be carried out in a far more satisfactory manner than by the standard machines hitherto used for the purpose. From the time of their installation, the mixers were used for both the mixing of bulk samples of seed and for the process of disinfection.

When it was found that Nomersan was not very effective for controlling seedborne *Phoma*, a search was made for more effective materials, and in the course of this work it was found that two proprietary products from the United States gave better results than Nomersan (Muskett & Colhoun, 1945*c*). These were New Improved Ceresan or Granosan containing ethyl mercury phosphate as its active constituent, and Arasan containing 50% tetramethylthiuramdisulphide as the fungicide. On account of its

poisonous nature it was decided not to use Granosan, but bulk supplies of Arasan were made available and have been used in Northern Ireland for the disinfection of the seed from the 1945 crop, while the discarding of seed contaminated with *Phoma* to the extent of more than 5% has been continued.

For the past three years a comprehensive health survey of flax seed produced in the United Kingdom has been made. Some 5000 seed samples have been examined annually by the Ulster method for contamination with *Colletotrichum linicola*, *Polyspora Lini*, *Botrytis* sp., *Phoma* sp. and *Fusarium Lini*. The results of this survey are of some interest in that they clearly show that flax seed produced in the southern and eastern districts of England is very significantly freer from contamination with seedborne parasites than that produced in the wetter parts of the British Isles to the west and north. Roughly speaking, the Pennines provide a convenient dividing line and it may be said that seed contamination is much higher to the north and west of the Pennines than to the south and east. At the same time, it is of interest to note that rust (*Melampsora Lini*) appears to be far more prevalent in crops grown from seed produced in the southern and eastern districts of England than from that produced to the north and west. The rarity with which *Fusarium Lini* has been found to occur as a seed contaminant clearly makes it of little significance as a seedborne parasite of flax in the British Isles.

Apart from the occurrence of *Phoma*, which did not appear to any extent until 1943, *Colletotrichum linicola* has been shown to be the most serious seedborne parasite encountered in the course of this work, and during the war period there has been a continuous build-up of this parasite in seed stocks produced in the north and west of the British Isles. At the same time, no epidemic of seedling blight in the flax crop occurred during the 1939–45 war, whereas it was responsible for heavy losses in 1914–18. This has undoubtedly been due to the general adoption of seed disinfection which is particularly effective against this particular disease, and it has been estimated from field experiments that such disinfection has benefited the flax crop during the war period to the extent of some £2,000,000. Two shillings and sixpence is the all-in cost of disinfecting sufficient seed to sow one statute acre.

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Reviews

Statistical Methods: Applied to experiments in Agriculture and Biology. Fourth edition. By GEORGE W. SNEDECOR. Pp. xvi+485. Ames, Iowa: The Collegiate Press, Inc. 1946. $4.50.

The statistician concerned with biological problems tends to divide biologists who lack statistical experience into three classes: those who know that all statistical concepts are so profound as to be completely beyond their understanding, those who know that statisticians deliberately make simple questions appear difficult, and those who know the statistical analysis of biological data to be unnecessary. The lucidity with which Prof. Snedecor develops his subject should encourage the faint-hearted, the variety of techniques he exhibits should confute the cynics, and the range of problems he subjects to statistical analysis should convert such of the heathen as trouble to read the book. The many research workers who have to acquire some facility in statistical technique, but who rarely have time to study the underlying theory, may find that R. A. Fisher's two well-known books, though giving a remarkably comprehensive view of the variety of biological problems in the analysis of which exact statistical processes are essential, are insufficiently detailed for the novice. To meet their needs many elementary texts have been published; often these have contained serious flaws of a kind that would pass uncorrected by the reader unacquainted with the theory of the subject, but, since its first appearance in 1937, Prof. Snedecor's has been one of the few that could safely be recommended to the inexperienced. The book has been to a great extent rewritten for the fourth edition, and contains much new material; though the general arrangement is unaltered, the presentation of the argument has been vastly improved.

A book whose purpose is to be a statistical 'cookery-book' rather than a theoretical treatise always introduces a risk that methods well suited to the analysis of one set of data may be completely misapplied by the reader in circumstances in which only greater knowledge of theory could have taught him their inappropriateness. That users of Prof. Snedecor's book should occasionally fall into this error is probably inevitable, but at least the cause will not be, as unfortunately it is for some books, the author's failure to make clear under what conditions a particular technique is valid. The first difficulty encountered by the writer of a book of this type is that he must not use advanced mathematical demonstrations, yet he must give more than dogmatic statements if he is to convince his readers of the practical relevance of the techniques he expounds. Prof. Snedecor overcomes this difficulty admirably by constructing empirically examples of the distributions required in the chief tests of significance. Thus in describing the χ^2 test he calculates values of χ^2 for a large number of random samples drawn from a binomial population, and shows how these generate a χ^2 distribution with one degree of freedom; he then explains how the hypothesis that a set of experimental results refer to a sample from a specified population may be tested by comparison of the value of χ^2 with this distribution. The empirical approach to tests of significance, later repeated for normal, t, and variance ratio distributions, is likely to give far greater satisfaction to the non-mathematician than pages of algebra, especially if he repeats some of the sampling experiments for himself.

The rapid development of exact tests of significance during the past 25 years has sometimes resulted in their being allowed to overshadow considerations of estimation. This tendency in its extreme form is evidenced by the not infrequent appearance of scientific papers plentifully ornamented with asterisks and comments that '$0{\cdot}05 > P > 0{\cdot}01$', but omitting to state the mean weights, heights, percentages, or other measurements to which these tests refer. Prof. Snedecor emphasizes that drawing from the sample of measurements which constitutes the experimental data inferences relating to the population from which the sample came is often of greater importance than mere classification of differences as 'significant' and 'not significant'.

The book is excellently suited to the needs of a biological or agricultural research worker without any previous experience of statistical techniques, for whose first reading a short course comprising about one-quarter of the book is suggested. The author rightly insists that mastery of the subject can only be attained through numerical practice, and in addition to the fully worked examples in the text he includes many others as exercises for the student; some of these are trivial, but all are well chosen to illustrate important points of theory and practice. The more experienced reader will also probably find much that is new to him, especially in such sections as those describing the χ^2 test for $2 \times 2 \times 2$ tables, the analysis of variance with disproportionate subclass numbers—a most useful account of several different methods—or the deletion of a variate from a multiple regression equation.

The early chapters lead the novice very gently from the results of sampling in binomial or normal distributions to the concepts of tests of significance and fiducial statements about the parent population. The χ^2 test for contingency tables and the t test for comparisons between two samples are explained and extensions of the uses of those statistics described. Linear regression is introduced in a manner which should make it understood by many who have previously found the idea difficult; the various forms of error that may be required for estimates based on the regression equation are very clearly described. Inclusion of a few paragraphs on the application of linear regression methods to biological assay would have increased the value of this chapter for many readers. A chapter on the correlation coefficient and its relationship to regression follows. Then come two chapters on analysis of variance which, though long, are more condensed than previous chapters and are consequently more difficult reading. These are amongst the most important

chapters of the book and could with advantage have been made still longer, for they scarcely give an adequate account of modern experimental design; nevertheless they contain detailed instructions for the chief forms of analysis of variance computations for equal and unequal subclass numbers.

Covariance and multiple regression are described in the next three chapters; the use of covariance analysis for improving the precision of experiments is well illustrated. The sections on multiple regression may intimidate some on account of the formidable array of symbols. The computations are quite simple providing that they are performed systematically, but Prof. Snedecor's account is perhaps unduly complicated by its presentation in terms of correlation coefficients; for most purposes, the reader would be better advised to work with sums of squares and products rather than correlation coefficients. The sections on orthogonal polynomials could have been simplified by writing them with reference to Fisher and Yates's *Statistical Tables* rather than to the original full method of calculation.

The chapter on individual degrees of freedom is perhaps the least satisfactory in the book, since it fails to impress the reader with the great power of factorial experiments for the investigation of complex biological phenomena. So far as it goes, it gives a good description of the technique of subdividing sums of squares, but scarcely mentions factors at more than two levels; the important device of confounding for the reduction of block size and consequently of experimental error is also not discussed. Perhaps Prof. Snedecor will eventually redress the balance by writing a second book on experimental design. After a further chapter on the analysis of data from binominal and Poisson distributions, including the use of transformations in the analysis of variance, the book ends with an excellent brief account of the principles underlying sample surveys; this again is a difficult chapter and one which, it is to be hoped, will be expanded in later editions, but it provides a good introduction to unstratified and stratified random sampling.

The book contains a useful collection of statistical tables, including, in addition to those usually found, a table of fiducial limits for binomial sampling, a table of range for normal samples, and others. Type and paper are good, the former being improved from previous editions. A number of misprints have been noted, but these are mostly too trivial to worry the reader.

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A Manual of Soil Fungi. By JOSEPH C. GILMAN. Pp. xi+392 with 135 text-figures. Ames, Iowa, U.S.A.: The Collegiate Press, Inc. 1945. $5.00.

Although fungi had been isolated from the soil by Adametz in 1886 soil mycology came to birth only with the researches of Oudemans & Koning in 1902 and reached adolescence with the studies of C. N. Jensen and Elizabeth Dale in 1912. Work in many countries during the next 15 years showed clearly that fungi are permanent inhabitants of all normal soils, that they occur in immense numbers and great diversity of species and that they play an exceedingly important role in the soil economy. In 1927 J. C. Gilman & E. V. Abbott published a *Summary of the Soil Fungi* in which 242 species were described, but this useful memoir soon became unobtainable and Dr Gilman has now more than replaced it by the present manual. In this nearly 700 species of soil fungi are described and another hundred of uncertain position are listed: even so the author has missed a number of recorded species, e.g. *Hyalopus crystallinus*, *Aspergillus oryzae*, *Pistillaria quisquiliaris*, etc., the last being of especial interest as it is one of the very few basidiomycete records.

The author purposely excludes from his treatment the 'terrestrial mushrooms, the plant pathogens which are considered to be soil-borne but which have not been isolated directly from the soil, and the forms which have been reported from leaf-mold, decayed wood or other substrates that have not yet become fully incorporated in the complex known as soil'. He also excludes the actinomycetes and the Zoopagaceae although he regards these as 'unquestionably important soil fungi', the first because of his 'lack of familiarity and the second because of the additional reason that these forms will not usually appear among the fungi that are found in plate cultures made from soil samples but require a special technique for their study'. He also omits the myxomycetes and yeasts; the former do not occur in ordinary soil plates but the latter are usually present in abundance. The author further recognizes that his manual is incomplete since he writes: 'Although the selective action of the medium used in the cultural work has thus limited the species treated in this manual, the forms presented are probably the predominant group in the soil.... The fact that the media used to-day for this type of investigation are rather well standardized, defines sharply the area in which the material in this manual will be useful.' I cannot help thinking that, in spite of his wide experience, Dr Gilman has not sufficiently recognized how partial his treatment may be. Numerous fungi present in any soil sample rarely or never appear in ordinary soil plates because, in addition to the selective action of the medium, the temperature of incubation or any other of the many cultural factors involved do not favour their growth. Furthermore, soil plates are quickly overgrown by the predominant species and many fungi that are inherently slow in germination and growth are simply occluded, or their germination and growth may be inhibited by the antibiotic and staling substances produced by the more rapidly growing forms. If these factors are eliminated by the adoption of special techniques there is a remarkable increase in the diversity of species that may be isolated from a soil sample. As the author remarks, however, 'the question of whether the fungus in the plate was in reality a soil inhabitant or merely the result of the accidental lodgment of a spore in the sample from which the isolation was made cannot be answered.' To my mind the evidence, so far as it goes, strongly suggests that the vast majority are true soil inhabitants.

Dr Gilman does not discuss the historical development or literature of soil mycology, the techniques of isolation and study, the numbers, the soil and biological relationships or the vital activities of the fungi; his manual is purely descriptive and is 'intended to be helpful in identifying molds already in cultures'. The book opens with a preface and introductory chapter and a key to the classes, orders and families of soil fungi. There follow the descriptions of species with notes of the countries from which each has been recorded and, inserted appropriately

in the text, are keys to the genera and species. The descriptions are well written and the salient characters of each genus are illustrated by text-figures. The volume closes with a bibliography of 169 titles, a glossary of 394 descriptive terms and an index of names. The geographic records, especially, should stimulate further enquiry since many species known only from one country must be present in others; thus, the only record of the interesting genus *Coemansia* is *C. reversa* van Tiegh. from Austrian soil, whereas this species as well as *C. pectinata* (Coem.) Bainier and *C. spiralis* Bain. occasionally appear in old dry plates of English grassland humus. Although it would have increased the length of the manual one cannot help regretting that in noting the countries the author did not also include the soil profiles.

In the soil a new world of life is opening up before us. In scale it is Lilliputian to our Brobdingnag, but this Lilliput is no dreamland or distant journey, it is the very soil beneath our feet. A crumb of soil is a world in microcosm, a world in which immense numbers and divers kinds of protozoa, algae, fungi, actinomycetes, bacteria, myxomycetes and perhaps viruses weave themselves into an endless and kaleidoscopic fabric of life. The fungi are no mean inhabitants of the soil, for upon their vital activities depend in great part the innumerable transformations that govern soil fertility, the growth of plants and the food of man. Many species of soil fungi are of basic importance in the manufacture of industrial products as also of their spoilage and, of recent years, they have given us the antibiotic substances. As yet the fungi of the soil have been little explored, but they present endless fascinating problems for our study, and in this endeavour Dr Gilman's manual should prove a useful tool.

WILLIAM B. BRIERLEY

Pflanzliche Infektionslehre. By ERNST GÄUMANN. Pp. 611 with 311 Text-figures and 90 Tables in the text. Basel, Switzerland: Verlag Birkhäuser. 1946. S.Fr. 48.50.

Those who treasure memories of plant hunting in 'the joyous Alps' find it easy to understand why Switzerland, a country only twice the size of Wales, with half the population of London, should have produced so many great botanists: 'Great things are done when men and mountains meet.' Eminent among Swiss botanists is our Honorary Member, Prof. Ernst Gäumann of Zürich. For a quarter of a century notable contributions to many aspects of plant science have streamed from his laboratory and he is recognized the world over as Magister in the fields of plant pathology and mycology. One need only recall his *Beiträge zu einer Monographie der Gattung Peronospora* of 1923; his *Vergleichende Morphologie der Pilze* of 1926 which, in C. W. Dodge's excellent translation, has become the mycological Bible of all English speaking students of the fungi; and the *Biologie der pflanzenbewohnenden parasitischen Pilze* written in collaboration with Prof. Ed. Fischer of Bern in 1929, an admirable volume which unfortunately has not received translation. And now there has appeared his *Pflanzliche Infektionslehre*, an illustrious work in which twenty years of thought, research and teaching have come to splendid fruition.

It is fortunate that from time to time in the march of science a scholar of deep insight and widely ranging mind brings together in one volume the whole scope of a particular field of enquiry and synthesizes it in such a way that divergent viewpoints and masses of data fall into place to create a unified and harmonious picture. Such a volume materializes the intellectual climate of the time and crystallizes the knowledge into sharp-edged form; it acts as a potent catalyst to workers in its own and adjoining fields and it serves as a master plan for the new era of scientific exploration to which it gives birth. These volumes are rare and still more rarely is it given in the life-time of one man to produce more than one such book, but Prof. Gäumann has achieved this distinction. His new work shows a far-reaching comprehension, a masterly handling of data and an elegance of treatment which derive only from a scholarly mind rich in the wisdom of experience and, running through its pages like golden threads, are a philosophic integration and a processional ordering that endow it with epic quality.

Further, the volume is a notable landmark in the development of the subject. Up to the present the study of plant disease has remained merely an increasing aggregate of data, techniques and expertize relating to its circumscribed area of natural processes and phenomena. It has not attained the status of a science, a 'universe of discourse', for such an aggregate only becomes a science in so far as it develops a consistent body of theory which correlates the facts into a logical and explanatory system. The importance of Prof. Gäumann's *Pflanzliche Infektionslehre* resides largely in the fact that it is the first serious attempt to develop the necessary theory. It is essentially a treatise on the theoretical foundations of plant pathology and it marks a long and decisive step towards the establishment of plant pathology as a science.

The volume is complete in itself but, at the same time, it overlaps and complements the earlier treatise on the *Biologie der pflanzenbewohnenden parasitischen Pilze*. This deals with the growth and life-relations of parasitic fungi existing in a particular habitat, the living plant. The fungi *qua* fungi occupy the centre of attention and the host plants and other environmental determinants have value only in so far as they provide the conditions necessary for the life of the parasite. In his new book the author makes a critical survey of the entire field of plant invasion by parasitic phanerogams, fungi, bacteria and viruses in relation to the conditions and mutual interactions of the host plants, parasite and environment. It is the relation itself that occupies the focus of attention rather than any one of the three elements involved in the relation, i.e. the state of disease is visualized as an integration at a higher level which subsumes its components. Moreover, infection is considered not as a problem of the special pathology of individual plant diseases but as a fundamental biological problem of the threefold interaction of the constitution of host plants, the invasive potentiality of parasites, and the conditioning factors of the environment. The structures and relations present in individual diseases have value in the author's consideration only as illustrations of the fundamental biological principles. Thus, in his trilogy, Prof. Gäumann has laid a splendid foundation for the understanding of the structure and reproduction of fungi, the life of parasitic fungi within their host plants, and the fundamental biological pro-

blems created by the host-parasite-environment relationship.

In his *Pflanzliche Infektionslehre* the author's treatment of the subject divides naturally into a sequence of six major topics, each occupying a chapter of the book. Chapter one deals with the manner of entry of a parasite into a host plant and its establishment in the tissues. Chapter two discusses the way in which parasites are brought into contact with their hosts, the influence of the environmental conditions and the epidemiology of infection. Chapter three considers the virulence and other parasitic qualities of invasive organisms in relation to the genetic constitution and development of the parasites and the conditioning influence of external factors. Chapter four, the longest in the book, discusses the receptivity and defence reactions of the host plant in relation to its genetic constitution and development and to environmental conditions. Chapter five deals with the processes and conditions underlying the morphological and physiological manifestations of the diseased state. Chapter six, a few pages only, indicates the prophylactic and therapeutic principles on which the control of disease is based. The last topic might, perhaps, be considered to lie outside the scope of the immediate problem but, although it receives brief attention, it is treated so suggestively that one is grateful for its inclusion. The first five topics are discussed in great detail and with such a wealth of cogent illustration that more than the bare outline of the author's treatment given above is impracticable in a short Notice. I can only say that I do not remember any other book in the literature of plant pathology that has left on me so strong an impression of the integrative quality of the author's mind, of his wide-ranging scholarship or of his powers of luminous exposition.

The book is excellently illustrated; it opens with a detailed and well-arranged Contents and closes with a splendid Bibliography and a full Index; and it is produced in a style worthy of its importance. It is number three of a series of textbooks and monographs on the exact sciences, being the first volume to be published in the section of experimental biology. It will be little short of a tragedy if this volume does not find rapid translation into English so that it may reach the widest possible circle of readers, for its influence on the development of plant pathology in our time should be incomparable.

WILLIAM B. BRIERLEY

Fungicides and their Action. By JAMES G. HORSFALL. Pp. 239. Waltham, Mass., U.S.A.: the Chronica Botanica Co. (London, W. 1: Wm. Dawson & Sons, Ltd.). 1945. $5.00.

James G. Horsfall has treated his subject in bold, downright fashion adopting without compromise the hypothesis that it is possible to analyse the reasons for the field efficiency of fungicides into those factors determining the amount of fungicide which comes into action and those factors determining the potency of that amount. It was perhaps during his stay at Long Ashton that the broad outlines of this theme were born, for it will not be unfamiliar to those who know the recent work of that Station on protective fungicides.

After a brief historical survey, Horsfall heads straight for the principles of chemical protection and of laboratory bioassay, the problems of deposition, coverage and tenacity. A chapter on immunization and chemotherapy precedes the development of the second part of his theme in which he discusses the mechanism of action of the copper and sulphur fungicides and treats, in a systematic way unexpected of a biologist, the synthetic organic fungicides. The book closes with chapters on joint action in fungicides and on phytotoxicity, while there is a well-balanced bibliography with general and author indices.

It is necessary first to become acclimatized to the breezy, colloquial style of the author who enjoys an apt simile. In places the reader is halted, as in the Preface, by an allusion to a 'fence row' between insect and fungal toxicology, and it is not until p. 9 that he can be sure of a correct interpretation of the phrase. But there is no explanation of why sulphur should be 'a "natural" as a pesticide' (p. 118). Nor is the description of spraying (p. 53) as a 'method of mixing a liquid with leaves by means of air and a prayer' helpful, for the jingle seems more applicable to atomization. More frequently the author's allusion is clear even when he is, on p. 32, 'dunking aphids in nicotine', i.e. dipping aphids into aqueous nicotine solutions. These bald examples give an impression of flippancy, but the general result makes for easy reading and, in most cases, the purpose of driving home a point convincingly is achieved.

Reviewing the subject matter as a whole it is questionable whether the author has covered the field of his title. Though reference to the use of fungicides outside phytopathology is frequent, the actual materials used in the mildew-proofing of wood, textiles and rubber receive, apart from the chlorphenols, scant attention, the reader being referred to a recent book on wood preservation. Within phytopathology the organo-mercury fungicides are dismissed, according to the index, in one sentence though their use in cereal-growing gives them front rank importance. But, with these exceptions, Horsfall treats his subject well and fully; his knowledge of the literature is deep, wide and detailed and his critical comments will be welcomed by the authors he cites. There are a few misprints, inescapable in a first edition, e.g. the initials and pagination of the reference to Brooks and Storey needs revision. There are a few historical mistakes as when, on p. 6, the reviewer is quoted as saying that Trillat first recognized, in 1888, the fungicidal properties of formaldehyde whilst, on p. 145, Trillat is credited with the discovery of this chemical. Formaldehyde was first prepared by Hofmann in 1867, first used as a disinfectant by Loew in 1888 and, for seed treatment, by Geuther in 1895; Trillat was associated with its commercial manufacture. A similar type of error occurs on p. 124 when the first description of tetramethylthiuram disulphide is dated 1934; but by this time this chemical was in commercial use for rubber vulcanization and the date is that of a patent protecting its use for fungicidal purposes. On the same page the dangers of the use of proprietary names are exposed, for these products are subject to inconstancy of composition while the trade name may be modified according to the requirements of the registering bodies in different countries. Thus Sulsol is, in this country, a paste sulphur preparation and not, as stated by Horsfall, compounded from the thiuram disulphide; Perronox (p. 109) is spelt here with one 'r'. Finally there are several chemical errors which, though not of

serious consequence to the chemist, may mislead or puzzle the student. To say, on p. 135, that pyridine is a constituent of nicotine implies that the latter is a mixture; the author intended to say either that nicotine is a derivative of pyridine or that the pyridine nucleus is a component of the nicotine molecule. The oxy-acids of sulphur quoted on p. 123 are wrongly described as 'reduced forms' of sulphur whilst it is curious that, in discussing the fungicidal properties of these acids on the previous page, Roach's work on the toxicity of thiosulphuric acid to *Synchytrium endobioticum* is not mentioned. The replacement of hydrogen in sulphanilic acid by ammonia does not give *p*-aminobenzene sulphonamide, as stated on p. 132, rather is the hydroxyl replaced by an amino group.

To have to point to defects in the book has been irksome but not, it is hoped, ungenerous, for their correction will render the book as valuable to the student as it already is to the more advanced worker. Horsfall has provided a brilliant survey of past work in his subject which he has enriched by pertinent and critical comments which reveal the master. Moreover, he is so far ahead in many sections that he has broken fresh ground as in the application of statistical units derived from the normal frequency curve to the correlation of properties well removed from the problem of bioassay for which they were originally developed. In discussing recent work on the search for synthetic organic fungicides he has pushed ahead on the 'toxophore' path with an enviable surefootedness. Every page, nearly every paragraph, contains a suggestion for further study and not one of his hypotheses is of the blind alley type which, by not being susceptible to practical test, serves only to encumber the work. It will be fascinating to watch, in future editions of this book, the development of the ideas with which it has been so generously enriched. Some may prove to be near-misses and will be modified; some will be verified and will remain as signposts guiding future workers beyond the regions in which Horsfall has been so able a pioneer.

H. MARTIN

Pyrethrum flowers: Supplement, 1936–45. By C. B. GNADINGER. Pp. 381–690. Minneapolis, Minn., U.S.A.: McLaughlin Gormley King Co. 1945. $5.

Since the second edition of *Pyrethrum flowers* appeared in 1936, Kenya has superseded Japan as the world's chief source of pyrethrum flowers and the large amount of information which has been made available during this period has been brought together in this volume. Ease of reference is facilitated by the fact that pagination of this supplement and numbering of new references (1372 in number) are continuous with those in the second edition of *Pyrethrum flowers* and the index includes a revised index to the second edition. The amazing development of the pyrethrum growing industry in Kenya is admirably described and includes an account of the investigation by the author and Mr V. A. Beckley (of the Kenya Department of Agriculture) which led to a guaranteed minimum pyrethrin content of 1·3% and contributed in no small measure to the rapid rise of the industry in the competitive market during the years immediately preceding the war. The relative importance of the flowers grown in other parts of the world and possible areas suitable for cultivation are discussed with great clarity, and the account should prove of value not only to scientific workers but also to those concerned with the development of suitable industries in various parts of the world and to farmers.

The recent chemical work which has led to the realization that pyrethrins I and II are complex mixtures is fully reviewed up to the beginning of 1945, together with the physiological action of the pyrethrins on cold-blooded animals and the effect on warm-blooded animals, with notes on cases reported of dermatitis due to pyrethrum products. Attention is drawn to various aspects of the work concerned with the mode of action of the pyrethrins on insects and the advances made in chemical and biological methods of evaluating toxicants, including pyrethrins, present in liquefied gases prepared for dispersion as aerosols. Newer methods of manufacturing pyrethrum powders and extracts and the stabilization of such products by the addition of antioxidants are reviewed. The extensive literature on synergists for pyrethrum is abstracted comprehensively and in addition, publications dealing with synthetic substitutes for pyrethrum, including the most outstanding at the present time—2:2:-bis (*p*-chlorophenyl)-1:1:1-trichloroethane (D.D.T.). A considerable section is devoted to the repellent action of the pyrethrins and a number of synthetic chemicals. There are a number of references to investigations on the suitability of various diluents in preparing powders containing pyrethrum and rotenone and the results obtained with pyrethrum containing insecticides against a wide range of plant pests. The importance of pyrethrum insecticides during the war is emphasized in the sections dealing with mosquito control and the aerosols. In these days of intensive and extensive research it becomes increasingly difficult to keep abreast of the literature, and in this book the author has reviewed judiciously and impartially. Throughout the book one feels that it has been written by an expert fully aware of the problems facing workers in this field and emphasis is made at the vital points. To quote just one example—after reviewing the work on aerosols, Gnadinger says 'the aerosol bomb is certain to bring about the development of more efficient sprayers' and in this short statement crystallizes the logical effect on peace-time investigation of this most interesting war-time method of utilizing the peculiar properties of pyrethrum to optimum advantage. The book is well printed on good quality paper and it is obvious that the proof was checked with great care. The volume can be recommended without reserve for the library of the worker concerned with methods of controlling insects.

T. F. WEST